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EFFECT OF FROST ON WHEAT AT PROGRESSIVE STAGES OF MATURITY

III. MILLING AND BAKING QUALITY[1]

BY R. NEWTON[2] AND A. G. MCCALLA[3]

Abstract

The yield of flour from unfrozen samples of wheat cut at progressive stages of maturity increased until the dry matter of the grain at the time of cutting reached 58%, and thereafter remained constant. Frost exposure reduced the flour yield at all stages of maturity, the reduction being roughly proportional to the immaturity of the sample and the severity of the exposure. The yield from mature, frozen samples was slightly but definitely lower than that from comparable unfrozen checks.

The baking quality of the unfrozen checks was relatively high, even when the wheat was cut while immature. Frost exposure reduced the quality of immature samples in proportion to the immaturity of the grain and the severity of the exposure, but had little if any effect on mature samples. Flour from immature, frozen samples deteriorated in storage more rapidly than did that from the unfrozen checks.

Reduction in flour yield was proportional to reduction in grade but reduction in baking quality was, on the average, less than anticipated from the grading results.

The earlier conclusion is confirmed that 58% dry matter represents a critical stage in the development of wheat, and that all samples harvested after this stage can be considered mature.

Introduction

Continuing the series of papers in which were described the physical characteristics of wheat kernels from plants which had been subjected to graded frost exposures at progressive stages of maturity in the seasons of 1929, 1930 and 1932 (7), and the composition and certain biochemical properties of the grain and flour from the 1930 samples (6), we now present the results of milling and baking tests of the 1930 samples.

A full description of the origin and treatment of the samples will be found in the first paper (7). They were all of the Marquis variety.

The 1929 samples, of four varieties, were also milled and baked experimentally, but notwithstanding that the frost exposures had been sufficient to cause in several instances a substantial lowering of the grade, as described in the first paper, no significant differences in flour yield or quality could be

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Contribution from the Department of Field Crops, University of Alberta, with financial assistance from the National Research Council of Canada. The co-operating laboratories of the Department of Agricultural Chemistry, University of Manitoba, and the Department of Chemistry, University of Saskatchewan, replicated the milling and baking of the composite samples. Issued as Paper No. 86 of the Associate Committee on Grain Research of the National Research Council and the Dominion Department of Agriculture.

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demonstrated. Laboratory milling by the equipment and methods so far available is admittedly an inadequate test for differences in milling quality that may be of real significance in commercial practice, and it seems likely that such differences actually existed, at least in those cases in which blistering was severe enough to reduce the weight per bushel (7). Laboratory baking methods and technique have, however, progressed to a point where we may with some confidence expect to detect any important differences in baking quality. The absence of demonstrable differences in the 1929 samples therefore suggests that even in those cases where the frost exposure led to a heavy cut in grade, the injury was little more than skin deep.

Profiting by this experience, we made the 1930 exposures more severe, and in the resulting samples it was possible to show chemical differences (6) and differences in both milling yield and baking quality. In addition to the results on the latter points, some data on the storage properties of flour from frosted wheat are included in this paper.

Previous Work

Although there have been numerous investigations on the effect of frost on the composition of wheat, there have been few which have included direct estimates of milling and baking quality.

References to the milling and baking value of frosted wheat are made in a number of the reports of the Dominion Grain Research Laboratory, two of which will be cited. In the first (2), it was reported that the most noticeable effects of heavy frost on the baking quality of flour milled from frosted wheat were a reduction in loaf volume and the production of bread which was gray or dark in crumb color. Results of individual tests indicated that when wheat was affected by only a slight bran frost, the quality was practically unimpaired. In the second report (3), it was stated that the frosted wheat samples of the 1929 crop studied had apparently been exposed to frost when they were nearly mature. This was advanced as the reason why the quality was not injured to the extent suggested by the outward appearance of the kernels.

Results obtained by Whitcomb, Day and Blish (8) indicated a higher value for frosted wheat than that with which it is usually credited, but did not warrant definite conclusions.

Whitcomb and Sharp (9) made a study of wheats from heads harvested at various stages of maturity, part of each collection being frozen artificially. On account of difficulties in milling which they point out, they drew no conclusion with regard to the effect of the frost on the flour yield of immature samples. They found no differences attributable to frost in the flour yields of relatively mature samples. The loaf volume was smaller and the color and texture of bread much poorer, when made from frosted, immature wheat than when made from the unfrozen checks, but these differences disappeared in samples which had been frozen while containing less than 46% moisture.

Johnson and Whitcomb (5) studied Marquis wheat frozen to varying degrees at different stages of maturity. They found that temperatures from 29° to 27° F. were low enough to cause damage to immature wheat. The stage of

maturity and severity of the frost determined the extent of the damage to baking quality. It was concluded that if wheat were frozen after the moisture content had diminished to 46 to 44%, *i.e.*, at or after the stiff dough stage, it was capable of producing as good bread as unfrozen wheat harvested at the same stage.

Geddes, Malloch and Larmour (4) reported the results of an extensive co-operative study of 228 samples of frozen and unfrozen wheat. The heavily frosted samples were harder to mill than the unfrozen or lightly frosted samples, owing to the tough and fibrous nature of the middlings. Flour yield decreased with grade. The percentages of bran frosted, heavily frosted and immature kernels were negatively correlated with flour yield. Baking quality was determined in three laboratories, using either a 55% patent or a straight grade flour, and four baking formulas. Absorption was higher in the lower grades. The average baking quality as measured by loaf volume, crumb color and texture decreased with grade, except in the instance of No. 4 which was superior to No. 3 Northern. Partial correlations showed that in estimating baking quality the various forms of damage need not be considered individually. On the whole, the grades given by official inspectors were logically related to damage found in this investigation. The anomalous relation found between No. 3 Northern and No. 4, the authors believe, would not be likely to recur, as the 1930 revisions of the Canada Grain Act were in line with their findings.

Milling Quality

Individual Samples

The individual samples of wheat described in the first paper of this series (7) were milled on an experimental mill, a straight grade flour (4) being produced. The results for all samples, expressed as percentages of the wheat, are presented in Table I, and those for the samples grown at the south end of the field

TABLE I

Flour yields of individual samples

Dry matter at cutting, %	Straight flour, % of wheat			Dry matter at cutting, %	Straight flour, % of wheat		
	8° frost	4° frost	Check		14° frost	10° frost	Check
31.2	40.7	47.7	46.1	34.0	36.1	38.4	52.5
34.9	44.5	52.7	53.4	38.1	39.7	40.8	57.9
39.4	49.9	57.4	59.6	40.9	35.9	36.3	58.5
43.8	44.2	57.7	62.2	45.5	43.2	43.3	63.8
46.8	53.2	61.4	64.6	46.7	48.0	47.2	65.4
48.3*	53.2	63.2	67.4	50.5*	50.6	50.6	65.5
50.5*	50.6	54.7	66.0	51.1*	49.2	—	62.7
51.1*	47.6	—	62.7	53.3*	50.6	50.2	66.4
51.3*	48.6	56.4	65.8	54.9	60.4	60.6	68.6
55.8*	51.0	56.0	68.6	57.4*	54.2	53.8	68.5
56.8	61.8	68.9	70.6	58.1*	61.4	—	69.2
57.3	65.1	68.4	69.8	58.5	66.3	65.8	70.0
58.1*	63.4	—	69.2	59.1*	61.6	—	68.0
59.1*	61.8	—	68.0	65.5*	66.0	—	67.9
65.5*	70.4	—	67.9	65.6	66.7	—	69.6
65.6	64.9	—	69.6	69.4	—	—	68.9

**Samples from north end of field.*

expressed as percentages of both the wheat and the total flour in Fig. 1. The results for south end samples only are included in these graphs because the other samples varied considerably in flour yields owing to the uneven ripening discussed in the first paper.

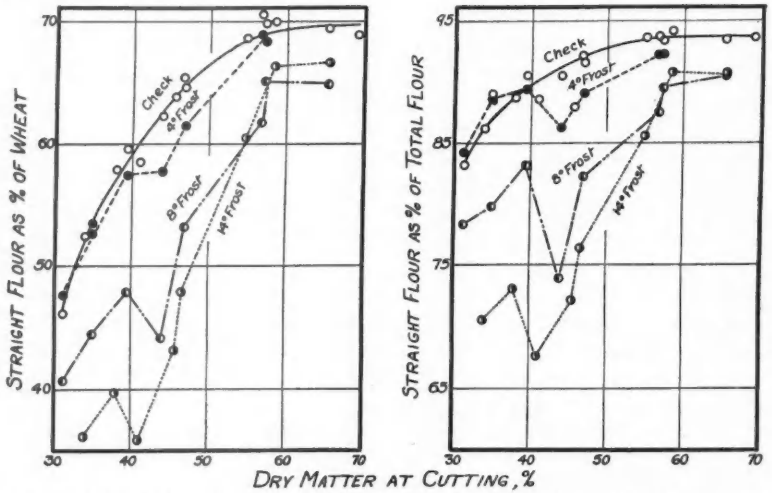

FIG. 1. *Flour yields of "south end" individual samples, subjected to various intensities of frost, in relation to maturity at cutting.*

The yield of straight flour from the unfrozen checks rose from 46 to about 69% of the wheat as the grain matured, until the dry matter content of the grain at cutting reached 56 to 58%. There were no significant differences among the later samples. The straight grade flour, calculated as a percentage of the total, increased with progressive maturity, and also reached a maximum in those samples containing 58% or more dry matter at the time of cutting.

In general, the flour yield from samples exposed to four degrees of frost was only slightly less than that from the comparable checks. The north end samples, which show quite heavy reductions as a result of this light frost, must be treated as a special case, because of their considerable admixture of green kernels. There were no differences in the two most immature samples, probably because immaturity caused almost as much shrivelling as was caused by the combined effect of immaturity and light frost. No mature samples were exposed to four degrees, but the two latest collections in this group (56.8 and 57.3% dry matter) were slightly reduced in flour yield, the blistering of the kernels apparently being sufficient to affect the milling quality appreciably.

Exposure to eight degrees of frost reduced the flour yield from immature samples markedly, and affected even the mature samples. Not only was there a reduction in the yield of straight flour, but there was also a larger proportion

of the total discarded as "feed". The yields reached a fairly constant level for all samples cut after the dry matter content reached 58%, but the level was approximately 4% lower than that reached by the unfrozen checks.

Exposure to 10 and 14 degrees of frost resulted in even more marked reduction in flour yields from the immature samples, but there were only slight differences between the results for comparable samples exposed to these two temperatures. There were no significant differences in the effects of the different exposures on mature wheat.

The difficulty encountered by Geddes, Malloch and Larmour (4) in reducing the middlings of low grade wheats was met with in milling the severely frozen samples of this series. The time required to mill a sample of the severely frozen wheat was considerably longer than that required to mill the unfrozen checks. Immature samples were more difficult to mill than mature ones, but immaturity was a less important factor than severe frost treatment.

Composite Samples

The material remaining after the individual samples had been studied was made up into composite samples as follows: Equal amounts of each of the five most immature check samples were thoroughly mixed and the mixture designated C1. Those samples exposed to four degrees of frost and falling within the first five collections were combined in equal quantities and this mixture designated C2. Similarly the samples harvested at the same stages and exposed to 8, 10 and 14 degrees of frost were composited and designated C3, 4 and 5 respectively. Similar composites were made up of the other material, the samples from each exposure and from the two ends of the field being kept separate. The complete series consisted of 31 samples made up as indicated in the first columns of Table II.

The grades of the individual samples have already been reported in the first paper of this series (7). The composites were graded independently and the results, which agree well with those for the individual samples, are included in Table II. These are referred to again in the general discussion at the end of this paper.

Portions of each of the composites were milled at the Universities of Alberta, Manitoba and Saskatchewan. The averages of the flour yields obtained at the three places are presented in the last columns of Table II, and those for the samples grown at the south end of the field in Fig. 2.

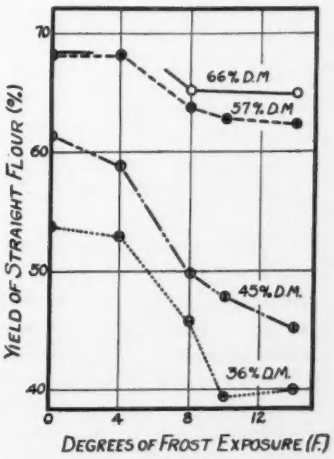

FIG. 2. *Flour yields of "south end" composites, of four stages of maturity, in relation to frost exposure.*

TABLE II

FLOUR YIELDS OF COMPOSITES

Sample No.	No. of individuals composited	Av. dry matter at cutting, %	Degrees of frost, F.	Grade**	Straight flour	
					% of wheat	% of total flour
C1	5	35.5	0	F	53.8	85.0
2	3	35.2	4	F	53.1	86.0
3	3	35.2	8	F	45.7	80.5
4	2	36.0	10	F	39.4	69.2
5	2	36.0	14	F	40.0	70.8
6	5	44.8	0	4	61.5	89.2
7	2	45.4	4	5	59.1	85.8
8	2	45.4	8	F	49.8	77.3
9	3	44.3	10	F	47.7	76.4
10	3	44.3	14	F	45.1	73.1
11*	6	50.8	0	5	64.0	88.6
12*	3	50.0	4	6	59.1	85.0
13*	4	50.3	8	F	47.0	75.6
14*	2	51.9	10	F	50.7	76.6
15*	3	51.6	14	F	50.7	76.5
16	4	56.3	0	2	68.3	91.2
17	2	57.0	4	4	68.3	90.4
18	2	57.0	8	5	63.8	87.1
19	2	56.7	10	6	62.9	86.4
20	2	56.7	14	6	62.3	86.1
21*	4	57.6	0	2	67.3	90.3
22*	1	55.8	4	F	56.8	81.6
23*	3	57.7	8	6	61.9	85.7
24*	1	57.4	10	F	56.8	81.3
25*	3	58.2	14	6	57.9	82.6
26	2	67.5	0	1	68.2	90.8
27	1	65.6	8	5	65.2	88.9
28	1	65.6	14	5	64.9	89.2
29*	1	65.5	0	2	68.4	91.2
30*	1	65.5	8	3	69.2	91.0
31*	1	65.5	14	6	66.8	89.2

*Samples from north end of field.
**F = Feed.

These results are in good agreement with those obtained with the individual samples. The yield of flour from the unfrozen checks reached a maximum for those samples cut at an average dry matter content of 57%. Four degrees of frost reduced the yield slightly, while 8, 10 and 14 degrees reduced it markedly except in the more mature samples. In all but one case (Sample C13) eight-degree exposures reduced the yield less than did exposures of 10 and 14 degrees. The yields from severely frozen samples cut when the average dry matter content was 57% were lower than those from the most mature, frozen samples. This result is not in disagreement with the conclusion reached as a result of study of the individual samples, because these composites included material frozen while at an earlier stage of maturity than the average

indicates, and such material yielded less flour when milled individually. The low yield of sample C22, exposed to four degrees of frost, can be explained by the fact that it was a single sample cut while containing less than 56% dry matter. It also was grown at the north end of the field, and much of it was greener than is indicated by the average dry matter content. While both the grade and the flour yield of this sample were lower than those of the composite (C23) made up of material exposed to eight degrees of frost, the flour yield was higher than that of any other sample grading "Feed".

The milling results as a whole show that both immaturity and frost exposure caused a reduction in the yield of flour as compared with that obtained from mature, unfrozen checks. The yield of the checks was little affected by the part of the field from which the sample originated, probably because the more immature kernels in the north end samples developed considerably after cutting. The question of such development has been discussed in the second paper (6) of this series. The gradient of reduction in flour yield by increasing severity of frost exposure is, however, steeper in the north end samples. Severe freezing checked translocation almost completely, and kernels which were immature when cut remained so. This reduced the flour yield in such unevenly ripened samples below that obtained from more uniform samples cut at the same average dry matter content. This relation is apparent in the results for both the individual and composite samples.

While both immaturity and frost exposure reduce flour yields, and therefore milling quality, the extent of the effect of frost is determined by the immaturity of the wheat and the severity of the exposure. The flour yield from immature wheat is reduced materially by frost treatment, while the yield even from mature wheat is slightly but definitely reduced.

Baking Quality

Individual Samples

The flour milled from the individual samples of wheat was baked, using the bromate formula (1, 4), one month and again 3½ months after milling. In appraising the quality of the bread produced, records were kept of water absorption, loaf volume, crust color, loaf form, crumb color and texture. The data obtained are too numerous to include in full, but loaf volumes and partial baking scores are presented in Tables III and IV. A full description of the method of scoring the various loaf characteristics is given by Geddes, Malloch and Larmour (4), who also give the method used in computing a single figure baking score. The partial baking score reported in this paper is calculated in the same way except that the score for loaf volume is omitted. The partial baking score is, therefore, a single figure estimate of the loaf characteristics other than loaf volume.

Water absorption is one of the values included in the partial baking score, and the fact that absorption is characteristically high in flour from frosted wheat masks the effect of the frost on the other values included in the score. To offset this, in certain cases indicated in the tables and figures the differences

between the absorption of the checks and that of the frosted samples were deducted from the partial baking scores of the latter, in order to put both on a comparable basis with respect to other characteristics.

The results for each character studied, while not presented in detail, have been summarized, and the differences between frozen and unfrozen samples are presented in Fig. 3. The results were divided into three groups: first, those for all samples harvested while containing less than 50% dry matter; second, those for all samples harvested while containing between 50 and 58%; and third, those for all the more mature samples. The results for the samples within each group and each frost treatment were averaged, and these averages used in plotting the curves in Fig. 3.

TABLE III

BAKING RESULTS OF INDIVIDUAL SAMPLES ONE MONTH AFTER MILLING

Dry matter at cutting, %	Loaf volume, cc.			Partial baking score			Partial baking score minus excess absorption	
	8° frost	4° frost	Check	8° frost	4° frost	Check	8° frost	4° frost
31.2	450	609	566	26	42	44	24	41
34.9	491	672	564	28	47	41	26	47
39.4	532	627	657	48	53	52	44	49
43.8	427	581	604	45	52	56	37	48
46.8	490	594	656	50	47	55	47	47
48.3*	488	547	590	38	52	53	33	50
50.5*	422	536	564	33	51	54	30	46
51.1*	506	—	593	25	—	55	20	—
51.3*	505	486	553	47	49	56	41	43
55.8*	594	554	598	43	46	55	39	44
56.8	612	672	628	46	57	54	43	55
57.3	622	618	606	56	55	54	54	53
58.1*	656	—	582	60	—	58	55	—
59.1*	644	—	616	58	—	62	55	—
65.5*	618	—	626	58	—	59	58	—
65.6	762	—	611	56	—	59	56	—
	14° frost	10° frost	Check	14° frost	10° frost	Check	14° frost	10° frost
34.0	310	336	601	25	22	42	19	18
38.1	322	324	596	22	25	49	12	12
40.9	412	406	622	32	35	55	24	23
45.5	426	448	624	41	48	55	30	36
46.7	537	524	668	46	55	53	37	45
50.5*	454	472	596	36	39	53	29	31
51.1*	460	—	593	27	—	55	24	—
53.3*	598	514	564	28	40	57	25	34
54.9	627	620	652	52	50	57	47	45
57.4*	620	572	535	47	50	57	46	46
58.1*	619	—	582	57	—	58	53	—
58.5	649	644	638	54	56	55	53	55
59.1*	569	—	616	57	—	62	55	—
65.5*	663	—	626	54	—	59	56	—
65.6	742	—	611	53	—	59	54	—
69.4	—	—	672	—	—	62	—	—

**Samples from north end of field.*

The samples falling within the third group were considered as being mature when cut, since in the study of physical (7) and chemical (6) properties, development appeared to cease by the time the dry matter content of the grain had increased to 58%. All other samples were considered as being immature when cut, and were divided into two groups only because the inclusion of the results for the more immature samples with those for the somewhat more mature, would inevitably result in a low average. For convenience they will be referred to as the "immature" and "intermediate" groups, though the latter is definitely immature. As this discussion progresses

TABLE IV

BAKING RESULTS OF INDIVIDUAL SAMPLES 3½ MONTHS AFTER MILLING

Dry matter at cutting, %	Loaf volume, cc.			Partial baking score			Partial baking score minus excess absorption	
	8° frost	4° frost	Check	8° frost	4° frost	Check	8° frost	4° frost
31.2	398	531	—	30	43	41	29	43
34.9	380	516	522	39	46	41	38	46
39.4	—	558	586	51	47	49	48	44
43.8	362	540	587	57	51	53	48	46
46.8	419	520	579	53	54	60	49	53
48.3*	470	536	542	45	53	56	40	50
50.5*	452	511	535	42	58	58	38	53
51.1*	396	—	564	44	—	54	35	—
51.3*	—	458	531	55	57	54	48	48
55.8*	525	518	570	39	45	56	37	43
56.8	480	597	600	51	59	54	45	52
57.3	582	659	623	56	54	54	55	52
58.1*	590	—	564	57	—	56	52	—
59.1*	570	—	534	56	—	57	53	—
65.5*	565	—	606	57	—	57	58	—
65.6	646	—	626	55	—	57	55	—
	14° frost	10° frost	Check	14° frost	10° frost	Check	14° frost	10° frost
34.0	262	282	450	21	22	36	15	16
38.1	298	324	562	20	24	50	11	12
40.9	338	353	580	28	31	56	21	21
45.5	365	375	568	40	51	60	31	42
46.7	414	347	563	39	44	55	29	33
50.5*	487	405	576	44	46	51	32	33
51.1*	—	—	564	40	—	54	37	—
53.3*	430	452	569	40	47	60	35	42
54.9	544	550	606	50	49	55	43	43
57.4*	564	522	542	33	33	56	31	31
58.1*	562	—	564	54	—	56	50	—
58.5	610	592	638	59	55	52	47	54
59.1*	560	—	534	55	—	57	53	—
65.5*	624	—	606	55	—	57	58	—
65.6	754	—	626	52	—	57	52	—
69.4	—	—	666	—	—	54	—	—

**Samples from north end of field.*

it will be apparent that in many respects the results for the samples in the intermediate group resemble those for the more immature, rather than those for the mature samples.

There were minor differences in the results of the first and second bakings. In general there was a slight decrease in the loaf volume of individual samples, the decrease being greater for immature than for mature, and greater for frozen than for unfrozen samples. There were but small changes in any of the other characteristics scored. The relative values for the various groups and exposures were unchanged as a result of the extra period of storage and, for this reason, the results for the two bakings have been averaged in plotting the curves in Fig. 3.

Loaf Volume

The results for the loaf volumes of the unfrozen checks were in general very uniform, there being comparatively small differences between mature and immature samples. The immature samples decreased somewhat more in loaf volume between the two bakings than did the mature, but the differences were relatively insignificant in most cases.

The loaf volumes of the samples exposed to four degrees of frost were similar to, but on the average slightly lower than those of the checks. The decrease in loaf volume between the two bakings of these lightly frosted, immature samples was only slightly greater than that of their checks. None of them were included in the mature group.

More severe frost exposure caused a marked reduction in the loaf volume of bread produced from immature samples. The volumes of bread from samples in the first group, harvested while containing less than 50% dry matter, were very low and in most cases the bread was of such poor quality as to be unsuitable for human consumption. The effects of 10 and 14 degrees of frost were more marked than those of 8, but 8 was sufficient to cause serious injury. The samples in the intermediate group were not injured as much, and there were no differences in the results for the 8-, 10- and 14-degree exposures (Fig. 3). In general, the effect of frost decreased gradually with increasing maturity of the wheat when frozen. Some of the riper samples of the intermediate group were apparently uninjured by the frost exposure, while the averages for the mature, frozen samples were slightly higher than that for the checks.

The increase in loaf volume due to frost exposure of the mature samples may perhaps be attributed in part to a slackening of the gluten indicated in the gluten scores already reported (6). It is well known that strong flours, fairly high in gluten content as these flours were, require a longer fermentation period than weaker flours for maximum development. It was found that the gluten of flour milled from unfrozen, mature wheat could be slackened considerably by blending the flour with 15% of another flour milled from severely frozen, immature wheat, and that the loaf volume was thereby somewhat increased. This affords one possible explanation of the apparent advantage

of the frozen over the unfrozen, mature wheat under the conditions of the test. Probably a more important contributing cause was the greater diastatic activity of the frosted samples (6). Certainly this was the predominant factor in the two samples, cut at 65.6% dry matter, which contributed most to the high average loaf volume of those frozen when mature. These two had a diastatic activity more than 50% greater than their check.

The loaves baked from severely frozen, immature samples were smaller at the time of the second baking than at the time of the first. Deterioration between bakings was more marked in these than in either unfrozen or lightly frozen samples. The mature samples which had been heavily frozen also deteriorated to some extent. The last result, however, was unduly influenced by the values for some of the samples. Others of the severely frozen, mature samples deteriorated little during the three months' storage.

Partial Baking Score

The partial baking score, which is a weighted average of the individual scores summarized in Fig. 3, gives a single figure estimate of all loaf characteristics except volume. Each of the individual characteristics will be briefly discussed before referring to the partial baking score.

The high absorption of the frozen samples has already been noted. Even the samples exposed to four degrees of frost appeared to be affected, and the effect became more marked with an increase in frost exposure. The samples exposed to 10 degrees of frost appeared to be higher in absorption than those

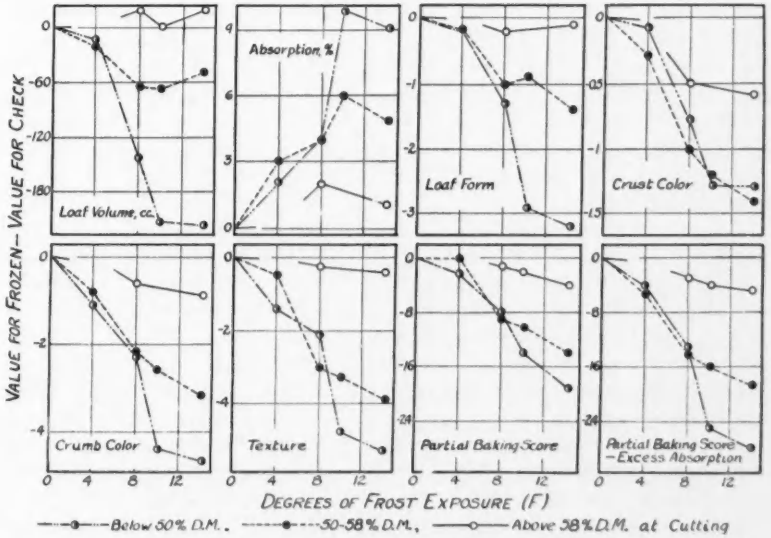

FIG. 3. *Average differences between baking quality of frozen and unfrozen individual samples, within three maturity groups, in relation to frost exposure.*

exposed to 14, but the differences were small and are unaccountable. The absorption of mature frozen samples was only slightly higher than that of the checks.

The results for loaf form show that the more immature samples were distinctly the poorest. The effect of frost increased with the severity of the exposure, but largely disappeared in mature samples.

The crust color of all samples was adversely affected by frost treatment. The immature samples were very high in diastatic activity, and were dark in external color. Many of the severely frozen samples were dull and mottled, the dull appearance being chiefly responsible for the reduction in the color scores of mature samples.

Frost treatment of the wheat affected also the color of the crumb of the bread adversely, the intensity of the effect depending on the maturity of the wheat and the severity of the exposure. The average score for samples of the intermediate group was more nearly like that for the samples of the immature group than like that for mature samples, indicating that the cause of injury to crumb color was operative until the wheat was practically mature.

The texture of the crumb is considered as the most important factor other than loaf volume in determining baking quality. The effect of frost on all immature samples was very pronounced, there being but small differences between the results for the samples in the two immature groups, and some of these differences favored the more immature one. The effect of frost practically disappeared in mature samples, a result in good agreement with those for most other characters studied.

The partial baking scores summarize the five factors discussed above. Except in the two most immature samples the differences between the partial baking scores of the mature and immature checks were very small. The frost affected loaf characteristics, other than volume, very adversely in practically all the immature samples, and the effect on the samples of the more immature group was only slightly greater than the effect on those of the intermediate group. In general, exposure to frost affected the quality of mature samples only slightly.

The defect in the partial baking score as applied to frozen wheat has been pointed out above, namely, that it gives undue importance to the abnormally high absorption of such samples. The curves for the partial baking score minus excess absorption indicate more fully the adverse effect of frost on baking quality than do those for the original score. Since the increased absorption of the frosted samples was proportional to the severity of the exposure, the reduction in their scores by subtracting this was in the same proportion.

The results for mature, frozen samples lend support to the suggestion that the larger loaf volume of a few individual samples must be taken, not as an indication of superiority in these samples, but rather as the result of other factors operating to allow a greater development of the frozen than of the unfrozen samples during fermentation. The lower scores for the frozen samples are an indication of weakness as compared with the unfrozen checks.

The baking results as a whole show that the effect of frost on immature samples was proportional to the immaturity of the wheat and the severity of the frost exposure. Mature samples were but little affected by the frost treatment.

Composite Samples

The composite samples were baked in three laboratories, using four different formulas. The bromate formula was used in all laboratories and the simple, malt-phosphate and blend-bromate formulas were used one in each laboratory.

TABLE V

Baking results of composite samples one month after milling

Approximate dry matter at cutting, %	Loaf volume, cc.					Partial baking score				
	Check	4° frost	8° frost	10° frost	14° frost	Check	4° frost	8° frost	10° frost	14° frost
*Bromate formula***										
36	629	667	614	332	325	46	47	44	20	20
45	660	661	528	488	507	53	55	45	41	40
51*	656	613	525	512	559	55	54	43	42	41
57	708	700	674	703	689	53	54	53	52	52
57*	683	613	718	615	693	56	54	51	46	49
66	812	—	843	—	854	54	—	53	—	54
65*	730	—	745	—	795	54	—	55	—	55
Simple formula										
36	530	478	560	418	330	36	33	39	20	18
45	541	457	452	494	620	43	37	44	45	48
51*	532	518	554	522	504	50	48	46	47	41
57	495	518	552	655	699	51	54	51	55	56
57*	509	575	588	639	688	51	52	51	54	54
66	548	—	603	—	694	55	—	56	—	55
65*	536	—	520	—	592	54	—	53	—	60
Malt-phosphate formula										
36	562	612	572	385	385	27	28	29	23	22
45	600	585	510	490	515	32	33	32	33	34
51*	605	598	582	582	510	31	35	32	32	32
57	640	652	660	660	700	41	44	36	41	41
57*	640	635	698	625	705	41	38	31	28	34
66	672	—	700	—	738	40	—	44	—	44
65*	678	—	668	—	738	40	—	42	—	45
Blend-bromate formula										
36	523	570	455	348	328	44	37	40	12	12
45	530	523	470	390	380	57	57	47	34	24
51*	540	500	430	423	415	57	51	36	29	23
57	578	553	538	543	545	51	52	51	54	52
57*	573	512	557	530	558	52	55	52	46	51
66	602	—	592	—	583	54	—	53	—	54
65*	575	—	585	—	570	54	—	55	—	53

*Samples from north end of field. **Averages of results from three laboratories.

The ingredients used in three of these formulas are described in papers published by members of the Associate Committee on Grain Research (1, 4). The blend-bromate formula used was the same as the bromate except that the flour was blended with 50% of a weak, soft-wheat flour. The results with the bromate formula obtained in the three laboratories were averaged, and these averages, together with the results obtained with the other formulas, are presented in Table V. The results obtained for the samples from the south end of the field with the bromate and blend-bromate formas are shown in Fig. 4.

The results obtained by the bromate formula, as might be expected, agree with those for the individual samples, baked by the same formula, and therefore require no discussion. The results obtained when the simple formula

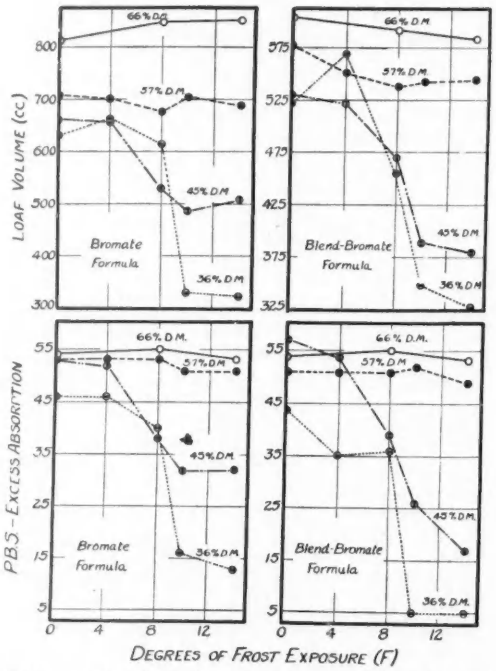

FIG. 4. *Baking quality of "south end" composites, of four stages of maturity, in relation to frost exposure.*

was used cannot of themselves be considered a satisfactory indication of the quality of the samples, because this formula does not provide conditions under which the stronger flours may develop to the full extent of which they are capable, while weaker flours may. The principal value of these results lies in the fact that from them the response resulting from the addition of bromate to the formula may be calculated and, since such addition may be considered

as equivalent to extending the fermentation time, a measure of the reserve strength of the flours obtained. The results for this response are presented in Fig. 5.

The immature check samples gave a greater response to bromate than did the immature samples exposed to 8 degrees of frost, while those exposed to 10 and 14 degrees gave slight negative responses. A discussion of the interpretation of such responses is given by Geddes, Malloch and Larmour (4). Flours giving a strong positive response are regarded as possessing reserve strength, those giving a low response usually show high baking quality by the simple formula and possess little or no reserve strength, and those giving a negative response are considered weak.

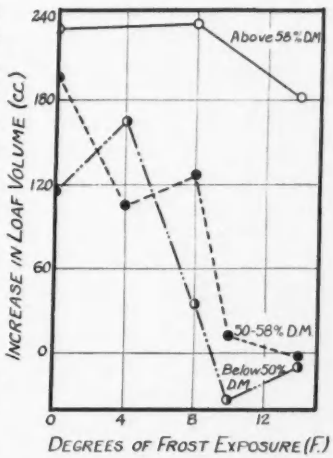

FIG. 5. *Bromate responses of composites, within three maturity groups, in relation to frost exposure.*

On this basis the more immature checks were stronger than the more immature frozen samples, with the possible exception of those exposed to four degrees of frost. The checks harvested while containing 50–58% dry matter were also stronger than the comparable frozen samples. Only in the mature group did the frozen samples exhibit as great reserve strength as the unfrozen checks. It seems possible, however, that other factors such as diastatic activity may have influenced the response to bromate. Nearly all frozen samples were higher in diastatic activity than their checks, and this would tend to increase the bromate response of those mature enough to escape serious frost injury to the gluten.

The use of malt in the malt-phosphate formula should have had the desirable effect of eliminating diastatic activity as a limiting factor in determining loaf volume. Loaves produced by the check samples using this formula were considerably larger than those obtained with the simple formula but smaller than those obtained when bromate was used. Mature, frozen samples appeared to be better than the mature checks, the addition of malt having caused an increase in the loaf volume of several samples even though the diastatic activity was already high. In experiments done subsequently to those now under discussion, Aitken and Geddes (1) found that either the malt-phosphate or the bromate formula gave increases in loaf volume over those obtained with the simple formula with samples which were fairly low in diastatic activity, but the increase by either formula was less than that when malt and bromate were both used. The interrelation of gas production and gluten development is discussed by these authors, who conclude that the use of an agent such as bromate is necessary to assist in the development of the gluten of only slightly aged, experimentally milled flours, and in addition,

conditions must be such that gas production is not limiting. In the present studies, the gas production of the mature checks was limiting when malt was not used, and the development of the gluten was inadequate when bromate was not used. Both of these factors were provided for when the bromate formula was used with the mature, frozen samples, so the results with this formula probably indicate the maximum volume obtainable with these.

The blend-bromate formula yielded results in almost strict accordance with expectation from the knowledge of the frost treatment given the various samples. The soft-wheat flour used in the blends was probably high enough in diastatic activity to insure adequate gas production, and would reduce the optimum fermentation period of the mature samples so that the checks were given the opportunity to develop to their full capacity. These results are in agreement with those for the bromate responses. Frost injury was thus revealed as proportional to severity of exposure and as declining with maturity.

Both loaf volume and partial baking score results show that with all formulas, but especially the bromate and blend-bromate (Fig. 4), the samples harvested while containing an average of less than 50% moisture behaved more like the most immature than like those harvested at an average dry matter content of 57%. There were small but apparently significant differences between the results for samples harvested while containing 57 and 66% dry matter, the latter being superior in all cases. The samples harvested at an average dry matter content of 57% included some material considerably less mature, so the results are not in disagreement with the general conclusion that the baking quality of the wheat was little if any injured by frost exposure after the grain had reached a dry matter content of 58%.

Storage Properties of Flour

The results with the individual samples had suggested that there was a more rapid deterioration of the flour from immature, frozen wheat than of that from the unfrozen checks. Flours from the composite samples milled at the University of Alberta were baked by the bromate formula 1, 7 and 14 months after milling, in the hope that more definite information on this point might be obtained. These samples had been stored at room temperature in tins not tightly sealed. In dealing with the results the samples were again considered in three groups, modified from those used previously (Figs. 3 and 5) in order to bring the north end series C11-15 (Table II) within the more immature group. The groups here are therefore: below 54%, 54–58.2% (to include Sample C25) and above 58.2% dry matter at cutting. The results for each group and each exposure have been averaged and are presented in Table VI. Unfortunately there was insufficient of some of the immature, frozen samples (indicated by asterisks in the table) to complete the third baking. These were the most immature of their respective sub-groups (represented by a single figure in the table), and if their results could have been present to influence the averages, the evidence of deterioration among the frozen, immature samples would undoubtedly have been even more emphatic.

TABLE VI

AVERAGE BAKING RESULTS OF COMPOSITES AFTER STORAGE

Time of storage after milling, months	Loaf volume, cc.					Partial baking score minus excess absorption				
	Check	4° frost	8° frost	10° frost	14° frost	Check	4° frost	8° frost	10° frost	14° frost
Samples under 54% D.M. at time of cutting										
1	619	605	572	495	495	56	56	49	37	36
7	548	544	502	398	407	51	54	45	33	26
14	514	580	409*	310**	285*	50	52	31*	36**	21*
Samples between 54 and 58% D.M. at time of cutting										
1	666	649	710	709	720	55	56	54	51	53
7	566	592	598	580	614	58	57	56	54	54
14	536	557	610	482	562	54	53	52	45	50
Samples above 58% D.M. at time of cutting										
1	758	—	825	—	870	57	—	53	—	53
7	726	—	760	—	698	56	—	55	—	54
14	683	—	674	—	612	58	—	58	—	55

**Only one of three original samples included.*
***Only two of three original samples included.*

There was some decrease with ageing in the loaf volume of samples in each group and at each frost exposure, but the check and four-degree samples of all three groups suffered less than did most of the other samples, and in no instance did the quality fall below the border line of that for acceptable bread. Exposure to 8 degrees of frost resulted in a deterioration of the immature sample, quite marked at 14 months, but the two groups of mature samples showed no greater deterioration than did the checks. Exposure to 10 and 14 degrees of frost induced still more rapid deterioration of the immature samples. There were some indications that the keeping properties of the more mature samples were also deleteriously affected by severe frost treatment. The loaf volumes at the time of the first baking were high, and although the volumes at the time of subsequent bakings were smaller, all of the mature samples produced bread which was acceptable.

In general the results show that the effect of relatively severe frost was so deleterious to the keeping properties of flour milled from immature wheat, that even samples which at first made satisfactory bread became unfit for use after several months' storage. The effect on mature samples was less definite, but the apparent superiority of the frozen samples discussed in earlier sections of this paper had entirely disappeared after the flour had been stored for 14 months.

Discussion

The general effects of maturity and frost on the milling and baking quality of wheat have been discussed in the previous sections. The main purpose of this supplementary discussion is to relate the results reported in this paper to those reported in the earlier papers of the series.

From the producers' point of view, the most important feature of frost damage is the effect on grade. We have shown (7) that even mature wheat was degraded when exposed to frost of varying severity. The decrease in grade was accompanied by a decrease in weight per bushel, but not by a decrease in weight per 1000 kernels. It seems likely that the effect on weight per bushel of mature frozen samples is related to the presence of blisters on the surface of the kernels. It is the presence of these blisters which establishes the fact that the sample has been frozen, and leads to the reduction in grade. The chemical studies (6) showed that the composition of wheat and of flour milled from it was not altered by exposure of the grain to frost after it had reached a dry matter content of 58%. The results of the chemical determinations carried out on the mature samples are not in agreement, therefore, with those for grade, but the stage of maturity represented by an average moisture content of 58% is established as critical in the development of the grain.

The milling results resemble those for grade. The average flour yield obtained from the samples of each grade except "Feed", regardless of the treatment accorded the particular samples included in that grade, are presented in the first section of Fig. 6. The average values plotted in the graph include the yields from both the individual and composite samples, the figure in brackets above each point being the number of individual results included in the average. Only one sample that was exposed to frost graded better than No. 4 Northern, so the results for No. 1, 2 and 3 Northern may be considered as applying only to the unfrozen checks. The yields of flour from

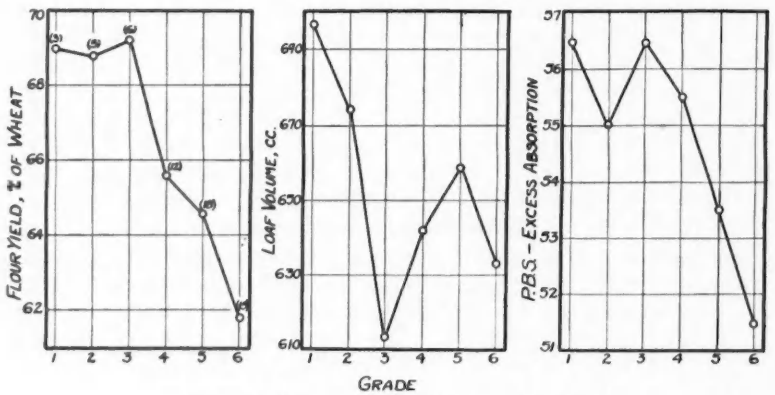

FIG. 6. *Milling and baking quality in relation to grade.*

samples of these three grades were essentially the same, but the effect of frost and immaturity on flour yield was approximately parallel to the effect on grade. These results are in agreement with those obtained by Geddes, Malloch and Larmour (4) with commercial samples.

The relation between grade and baking quality as measured by loaf volume and partial baking score minus excess absorption is shown in the other sections of Fig. 6. The values plotted are averages obtained with the bromate formula for both individual and composite samples. There was greater variation in the loaf volumes of unfrozen checks grading No. 1, 2 and 3 Northern than in those of frozen and immature samples grading No. 4 Northern, No. 5 and No. 6. The average volume of 14 samples grading No. 3 or better was 647 cc., while that of 45 samples grading from 4 to 6 was 646 cc. The comparable averages for partial baking score minus excess absorption were 56.0 and 53.8. These averages are influenced, of course, by the inclusion of results for samples frozen when relatively mature, but 42 of the 59 samples were immature when harvested. While these results indicate that injury to baking quality was not proportional to reduction in grade, one cannot deduce such a conclusion definitely because the baking formula used did not ensure adequate gas production in the higher grades.

These results are not in good agreement with those presented by Geddes, Malloch and Larmour (4), who found that loaf volume in general decreased with grade. Results obtained with other frozen samples suggest that if the protein content of two frozen samples grading No. 6 is, for example, 10 and 15%, the low protein sample will show the effects of the frost very markedly, while the high protein sample may yield good bread. In such cases the higher protein content allows the dough to take fuller advantage of the higher gas production in frozen wheat flour. All of the samples used in the present study were higher in protein content than the average of those studied by Geddes, Malloch and Larmour, and it is possible that this is one reason for the disagreement in results.

The gluten studies (6) indicated that the quality of the protein even of mature samples exposed to frost was slightly reduced. If the relatively mature frozen samples owed their high quality at least in part to the quantity of protein, which afforded sufficient strength to obscure the limited effect of frost on the quality of the protein, the discrepancies between the results for the baking and gluten studies can be reconciled. The indicated deterioration of the heavily frozen, mature samples in storage is of course in line with the observation that the frost had damaged the gluten.

In general, the baking results resemble those obtained in the chemical studies, that is, there was little apparent effect of frost after the dry matter content of the grain exceeded 58%. Although there are indications of slight injury to the baking quality of mature samples, this injury was less than anticipated from the decrease in grade.

The quality results as a whole support the conclusion that the development of wheat is largely completed by the time the dry matter content of the grain reaches 58%. Although frost reduced the grade, weight per bushel and flour yield of more mature samples, the effect of the frost was restricted largely to the outer layers of the kernels.

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GENERAL PRELIMINARY STUDIES ON THE PHYSIOLOGY OF DELAYED GERMINATION IN *AVENA FATUA*[1]

By L. P. V. Johnson[2]

Abstract

A series of general preliminary studies was made on the physiology of delayed germination in *Avena fatua*, the results of which may be summarized as follows: (1) Great variations were found in the after-ripening periods of a number of *A. fatua* selections. (2) Evidence was obtained which strongly indicated that delayed germination is determined by a condition of the seed coat which develops after fertilization. (3) Results from tests of entire panicles indicated a correlation between germinability and the position of the seed in the panicle. (4) The after-ripening period of secondary grains was shown to be much longer than that of primary grains. (5) The placing of incompletely after-ripened grains under germinative conditions induced secondary dormancy. (6) Exposure to light appeared slightly to stimulate germination in seeds which were in the early stages of after-ripening, but appeared to have a harmful effect upon seeds which were more or less completely after-ripened. (7) Low dry-storage temperatures retarded the after-ripening process. Storage in a frozen condition at freezing temperatures resulted in increased germination. Seeds moistened and subjected to outdoor conditions failed to germinate. (8) Dormancy was more or less completely overcome by breaking the seed coat over the embryo, or by soaking seeds in potassium nitrate solutions. The exposure of seeds under germinative conditions to an atmosphere having an increased oxygen concentration definitely stimulated germination. Treatments with pure oxygen, ether, and sodium thiocyanate had more or less indifferent effects upon germination, while ethylene chlorhydrin and dichlorethylene were definitely injurious.

It is inferred from the combined results that delayed germination is due to post-fertilization changes, related either to tissue absorption or development, which occur in the seed coats of *A. fatua* but not in readily germinable species, and which result in a restriction of the oxygen supply to the embryo. It is believed that the after-ripening process may consist, essentially, of a series of changes in the tissues of the seed coat which result in an increased permeability to oxygen.

Introduction

The studies reported herein were undertaken originally with the view of providing a physiological background for an investigation on the inheritance of delayed germination in hybrids of *Avena fatua* L. and *A. sativa* L. Later, however, they were extended to include physiological phases of the problem which had no direct bearing on the genetical work and, as now reported, are considered as general preliminary studies on the physiology of delayed germination in *A. fatua*.

A. fatua, the common wild oat, possesses the property of delayed germination, that is, its seeds will germinate only after a lapse of from several months to a year or more after harvesting. Certain physiological phases of this property were studied by Atwood (1) who reported results which indicated

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that in freshly harvested seeds, oxygen supply was the limiting factor to germination, and that seed coat restriction to oxygen entry probably played a role. The question of the nature of the physiological processes for which oxygen is essential was left open. Garber and Quisenberry (7) also observed that the germination of freshly harvested *A. fatua* seeds was increased by the breaking of the seed coat. Apart from the work of Atwood and of Garber and Quisenberry, no experimental results have been reported, as far as is known, on the physiology of delayed germination in *A. fatua*.

The present work has included the following phases of the problem: comparison of delayed germination in several selections of *A. fatua;* anatomical and physiological bases of delayed germination; relation between position in the panicle, maturity and germinability of seeds; after-ripening periods of primary and secondary grains; development of secondary dormancy; effect of light upon germination; effect of temperature upon germination; and effect of chemical treatments upon germination.

Materials and Methods

Practically all materials trace back to 17 *A. fatua* plants which were selected in 1933 from the experimental fields of the University of Alberta, Edmonton, Alberta, Canada. A few tests were made on the seeds of the original selections, but in most cases material grown separately from each of these plants was used.

The original selections were designated by the letter F followed by the numbers 1 to 17, respectively. Selections from the progeny of the original selections were identified by a number in parentheses. Thus, F-6 (1) represents the first selection from the progeny of original selection number six.

Fourteen of the 17 original selections were typical *A. fatua* plants, two (F-2 and F-15) were selected because of particularly large seeds, while one (F-16) was selected because of a reddish lemma color which differed distinctly from the usual dark brown.

Germination tests, unless otherwise noted in the text, were made in a thermostat-controlled, electrically heated germinator held at 20° C. Only large, mature, sound, primary grains were used, except in the case of special tests of secondary grains. The seeds were tested between the folds of paper pads placed on large blotters which fitted the germinator trays. These pads were easily and cheaply made from white paper towels and proved very satisfactory. Unless otherwise noted, tests were made on 50-seed lots from individual plants, placed in separate pads, and continued for ten days. Where special methods were used, the details are given in the sections of the text devoted to the tests concerned.

Where several test lots were drawn from a single sample, they were counted out by a method designed to eliminate the possibility of selecting the best seeds for a given lot. Such a method was necessary because of the practice of choosing only large, mature, primary grains from the seed stocks of individual plants which were often quickly depleted of the type of seed being

selected. The method used may be illustrated by the following example: If four lots of 50 seeds each were to be taken from a single sample, they would be counted out, five seeds at a time, in the following order:

1, 2, 3, 4,
5, 6, 7, 8,
9, 10, etc.

When 40 five-seed groups had been counted out in this manner into four rows of ten groups each, each of the four rows would be bulked separately to give the required four lots of 50 seeds each.

Unless otherwise noted, all material was stored continuously in the laboratory at room temperatures and tested between four and seven months after harvesting.

The reliability of the method of selecting and testing was checked as follows: Ten lots of 50 seeds each were selected from F-6 (bulked) material and tested for germinability (eight months after harvesting) as outlined above. The percentages of germination obtained were, successively, as follows: 24, 20, 16, 18, 14, 12, 14, 16, 20, and 14; the average being 16.8%. These results show that, under the conditions of testing, small differences in the germination of single lots of seeds are not significant. The degree of constancy in this test is, however, believed to be sufficiently good to permit reasonable confidence in the results reported in the present paper.

Experimental Results

Comparison of Delayed Germination in Several Selections

Fifteen selections (F-1 to F-15 inclusive) were tested for germinability in relation to the time elapsing after harvesting. Results from these tests, together with comparisons with the seed weights of the different selections, are given in Table I.

TABLE I

DIFFERENCES IN GERMINABILITY OF *A. fatua* SELECTIONS AT DIFFERENT STAGES OF AFTER-RIPENING, AND COMPARISONS WITH SEED WEIGHT

Material	Weight per 50 seeds, gm.	Germination, %		Material	Weight per 50 seeds, gm.	Germination, %	
		After 5 mo. (approx.)	After 18 mo. (approx.)			After 5 mo. (approx.)	After 18 mo. (approx.)
F-1	1.18	0	46	F-9	0.99	0	6
F-2	1.46	60	100	F-10	1.15	0	50
F-3	1.08	0	74	F-11	1.19	0	92
F-4	1.14	0	12	F-12	1.21	10	80
F-5	1.08	0	0	F-13	1.21	0	45
F-6	1.21	2	78	F-14	1.18	0	80
F-7	1.11	0	50	F-15	1.48	58	100
F-8	1.13	0	54				

These results indicate that there are great differences in the germinability of different selections after five months of after-ripening, that these differences still exist after 18 months of after-ripening, and that there appears to be a positive correlation between weight of seed and germinability at both periods of testing. The correlation between weight of seed and germinability after 18 months was found to be $r = 0.733 \pm 0.081$. Seed weight was based upon 50 primary grains with awns removed. This correlation may be due to natural hybridization between *A. fatua* and the heavier-seeded, readily germinable *A. sativa* forms.

A series of tests was also made at monthly intervals for the purpose of determining the time when germinability commences and the rate of the change from a completely dormant to a completely germinable condition. The results of these tests are given in Table II.

TABLE II

PERCENTAGE GERMINATION IN A SERIES OF TESTS MADE AT MONTHLY INTERVALS

Material	Percentage germination at monthly periods after harvesting						
	2 mo.	3 mo.	4 mo.	5 mo.	6 mo.	7 mo.	8 mo.
F-7(1)	0	0	12	12	12	14	24
F-9(1)	0	0	0	2	2	4	—
F-15(1)	0	0	20	28	22	40	40

The results indicate that the period of complete dormancy begins to disappear at about four months after harvest for the more germinable types and later for the less germinable types. After commencing, germinability does not show a steady increase but appears to level off for a few months and then to increase again. Unfortunately, the limited seed supply prevented the extension of the test beyond the eight-month period. Seeds from four *A. fatua* plants selected from the experimental fields at the State College, Pullman, Washington, failed to germinate in any of the several successive tests made between two and eight months after harvesting.

Anatomical and Physiological Bases of Delayed Germination

Atwood (1) concluded that the delay in germination of *A. fatua* seeds was occasioned by a restricted oxygen supply to the embryo due to the obstruction of oxygen entry by the "seed coat." This conclusion infers that the direct cause of delayed germination lies in a condition of non-embryonic tissue.

In the present work, the question of whether the direct cause of delayed germination was embryonic or non-embryonic in nature was attacked from three angles: (*i*) by germination tests of hybrid (or F_0) seeds arising directly from crosses between *A. fatua* and *A. sativa* in which *A. fatua* was the maternal parent; (*ii*) by observations on the germinability of seeds from hybrid plants; and (*iii*), by experiments in which *A. fatua* seeds were dehulled and variously treated with respect to the "seed coat" (pericarp and testa).

(*i*) Several crosses were made between a number of *A. fatua* lines [F-6(2), F-9(2), and F-15(1)] and *A. sativa* var. Victory in which the former were the maternal parents. Twelve hybrid seeds were produced in this manner. (The variety Victory is capable of complete germinability soon after harvest). As germinability is the dominant character in such crosses (7), the effect of crossing is actually the placing of germinable embryos within the extra-embryonic parts of *A. fatua* seeds. In all cases, seeds arising directly from crossing germinated when tested 75 days after harvesting, while *A. fatua* parental checks did not germinate. The germinability of the hybrid seeds cannot have been due to the effect of tissues developed prior to or independently of fertilization, since such tissues would be the same in the *A. fatua* parent. It must be concluded, therefore, that germinability was brought about through the influence of the *sativa* elements in the zygote, the inference from which is that any tissues concerned must have been developed after, or influenced by, fertilization.

Experiments were conducted to determine the effect of emasculation and pollination upon the germinability of *A. fatua* seeds. Florets were opened as in emasculation, one anther removed from each and the panicles covered, as in the actual hybridization work, with a glassine bag. Two or three days later the florets were again opened, simulating the mechanical part of pollination, and the bags replaced. This procedure was found not to have stimulated germinability when germination tests were made subsequently upon 50 seeds.

(*ii*) Further support for the above conclusions is afforded by the fact that segregation for germinability was observed among the seeds of single plants in the F_1 and other generations from crosses between *A. fatua* and *A. sativa*. The only possible explanation of such segregation lies in the assumption that the recombination of genetic factors brought about by the self fertilization of each floret had an immediate influence upon germinability—which can only mean that germinability is determined by tissues or other causal agencies developed after, or influenced by, fertilization.

(*iii*) Results from the third experiment provide additional information regarding the factors determining germinability.

Seeds from a number of selections were dehulled with great care by a process of peeling the hulls from the caryopses. Where the dehulled seeds were to receive no further treatment, they were dropped gently from the hulls directly to the germinator pad. The dehulled seeds were given three different treatments: one group of seeds was rolled with considerable force between the thumb and forefinger, another was scraped over the embryo with a razor blade, and a third group received no treatment except very careful dehulling. The results of germination tests of the variously treated seeds are summarized in Table III.

The tests of F-1, F-2, F-3, F-4 and F-5 were made in 1933, 20 seeds being used for each test. In 1934 an attempt was made to repeat the experiment

on a larger scale; but, because of high germinability of the remainder of the material tested, only F-13(3) gave significant results. The results from F-13(3) are included with the previous data in Table III.

TABLE III

PERCENTAGE GERMINATION OF A NUMBER OF *A. fatua* SELECTIONS AFTER VARIOUS TREATMENTS OF THE HULL AND SEED COAT

Material	No. of seeds per test	Treatment			
		Hulled	Dehulled	Rolled	Scraped
F-1	20	0	0	45	—
F-2	20	60	60	100	—
F-3	20	0	0	—	100
F-4	20	0	0	—	100
F-5	20	0	0	85	—
F-13(3)	50	0	6	30	56

It may be concluded from the data in Table III that very careful dehulling (removal of lemma and palea from the caryopsis) has no appreciable effect upon germinability; but that general injury to the cuticle, pericarp and testa (as by rolling), or definite breaking of these structures over the embryo, results in a marked stimulation of germination. These results, considered in the light of previous conclusions, would indicate that delayed germination is brought about by the tissues covering the embryo, the character or development of which is influenced by the genetic recombinations resulting from the fertilization of the floret in question.

It is known that after fertilization great modifications take place in the tissues enveloping the embryo. Robbins (13) states that before fertilization the enveloping tissues of the oat seed consist of the following, named successively from the outer to the inner: outer epidermis, parenchyma layer, chlorophyll layer, inner epidermis, outer integument, inner integument, and the nucellus. After fertilization, all of these tissues are more or less completely absorbed, with the exception of the outer epidermis and the inner integument. In the mature seed, the outer epidermis with remnants of the parenchymatous layer forms the pericarp, while the inner integument becomes the testa. The pericarp and testa are fused to form the "seed coat".

It is concluded on the basis of the above results and discussion that delayed germination in *A. fatua* is due to agencies, operating after and influenced by fertilization, which affect the development or absorption of tissues enveloping the embryo in such a manner as to prevent germination of the seed until after a certain period of after-ripening has elapsed.

Relation between Position in the Panicle, Maturity and Germinability

The possibility of germinability being affected by the position of the spikelets in the panicle was investigated, together with observations on the effect of seed maturity.

The F-12 line was selected for study because of its intermediate degree of germinability (see Table I.). It was attempted to procure a panicle in which all spikelets were completely ripened and still retained in position, by means of placing the panicle between screens which prevented the dropping of mature grains. Unfortunately, when the panicle so treated was tested only one seed germinated.

Several other panicles at various stages of maturity were harvested from the progeny of Selection F-12, the most mature being at the stage when the upper seeds had begun to drop. Germination tests were made five and one-half months after harvesting.

In a general way, all panicles showed the same manner of germination with respect to panicle position, regardless of the stage of maturity; that is, seeds in "germinable positions" in immature panicles germinated even though less mature than seeds in "non-germinable positions" in mature panicles. Table IV serves to indicate the relation between the point of occurrence in the panicle and the germinability of the seed in a typical panicle of the most mature type. The "shelled out" seeds mentioned in the table were those dropped from the panicle in handling, and were for the most part from the tips of the longer branches of the upper whorls.

TABLE IV

RELATION OF POSITION IN THE PANICLE TO GERMINATION OF PRIMARY AND SECONDARY FLORETS OF F-12(2)

	Lower whorl		Second whorl		Third whorl		Upper whorls		Seeds shelled out	
	Pri.	Sec.	Pri.	Sec.	Pri.	Sec.	Pri.	Sec.	Pri.	Sec.
No. of seeds	13	12	13	10	15	10	19	8	7	2
No. germinated	2	0	3	0	9	0	13	0	6	0
Germination, %	15.4	0.0	23.1	0.0	60.0	0.0	68.4	0.0	85.7	0

It will be noted that there was a progressive increase in the germinability of primary grains in passing from the lower to the upper, earlier-maturing whorls. Where seeds from lower whorls germinated, they were in all cases from the tips of the longer branches, the points of earliest maturity for the whorls in question. Thus, in a given panicle, position and maturity were correlated; and both, in turn, were correlated with germinability. The germination of relatively immature seeds in the upper whorls and branch tips in the two immature panicles tested would indicate that position and not maturity was the factor primarily correlated with germinability.

It will be observed that in no case did secondary grains germinate.

In *A. sativa* var. Victory it was found that very immature seeds, both primary and secondary, germinated almost as well as fully matured seeds, regardless of position in the panicle.

After-ripening Periods of Primary and Secondary Grains

In the study of germinability in relation to the position of seeds in the panicle, it was found that secondary grains did not germinate, a fact which led to an investigation of possible differences in the length of the after-ripening periods of primary and secondary grains.

After individual-plant material was selected, all spikelets with both primary and secondary seeds intact were examined. Those with both grains sound and reasonably mature were selected and the primary and secondary grains separated for testing. Fifty primary and 50 secondary seeds were also selected from the threshed-out seed of each plant. The results from tests made seven months after harvesting are presented in Table V. Relatively lower germination was obtained from seeds of the selected spikelets, since the spikelets retaining both grains intact tended to be less mature and from lower positions in the panicle than the threshed-out seeds.

TABLE V

DIFFERENCE IN GERMINATION OF PRIMARY AND SECONDARY GRAINS SEVEN MONTHS AFTER HARVESTING

Material	Seeds from same plant			Seeds from same spikelet		
	No. of seeds each sample	Germination, %		No. of seeds each sample	Germination, %	
		Pri.	Sec.		Pri.	Sec.
F-6(4)	50	0	0	33	0	0
F-12(2)	50	16	0	25	12	0
F-15(1)	50	48	0	20	30	0
F-15(6)	50	58	0	20	30	0
Ave. germination, %	—	30.5	0.0	—	18.0	0.0

These data show unmistakably that there are very marked differences in the germinability of primary and secondary grains. However, such differences might be due to smaller size or other factors which may tend to lower the vitality of secondary grains. If the lack of germination were due to true

TABLE VI

DIFFERENCE IN GERMINATION OF PRIMARY AND SECONDARY GRAINS 18 MONTHS AFTER HARVESTING

Material	Seeds from same plant			Seeds from same spikelet		
	No. of seeds each sample	Germination, %		No. of seeds each sample	Germination, %	
		Pri.	Sec.		Pri.	Sec.
F-2	50	100	84	15	100	93.3
F-6	50	78	20	—	—	—
F-10	50	50	0	—	—	—
F-12	50	80	40	10	100	30
F-15	50	100	82	10	100	100
Ave. germination, %	—	81.6	45.2	—	100	74.4

dormancy rather than to low vitality, it would be expected that after a more extended period of after-ripening, the secondary grains would show a capacity to germinate. The test was, therefore, repeated using older material, including seeds from the progenitorial plants of the material used in the first test. The results from the second test in which the material used was 18 months old are given in Table VI.

The second test shows that secondary grains are capable of high germinability when given a sufficient period of after-ripening.

It is concluded from the results of the two tests that secondary grains of *A. fatua* require a much longer period of after-ripening than primary grains. The survival value in nature of such a difference would lie in the fact that the seed from a given plant would provide propagules for at least two successive seasons.

A similar adaptation is found in *Xanthium*, the cocklebur, where the so-called lower seed germinates the first spring after maturity while the upper seed remains until the following spring or even later before germinating (4).

Development of Secondary Dormancy

The almost complete lack of germination in re-tests of dried, non-germinated seeds from previous tests made it quite obvious that the placing of insufficiently after-ripened seeds under germinative conditions caused the development of a form of secondary dormancy. Secondary dormancy has been observed in the seeds of a number of other species (3, 10). The term is used to distinguish between an induced dormancy and the primary dormancy present at maturity in the seeds of certain plants.

In order to study further the matter of secondary dormancy in *A. fatua*, seeds were retained from tests made about four months after harvest, stored in a dry condition for three months, and then again tested, together with continuously dry-stored seeds from the same respective seed stocks. The results of these tests are given in Table VII.

TABLE VII

DATA INDICATING THE DEVELOPMENT OF SECONDARY DORMANCY THROUGH PLACING INSUFFICIENTLY AFTER-RIPENED SEEDS UNDER GERMINATIVE CONDITIONS

Material	Tested 4 months after harvesting		Non-germinated seeds from previous test, re-tested 3 months later		Continuously stored seeds, tested 7 months after harvesting	
	No. of seeds	Germination, %	No. of seeds	Germination, %	No. of seeds	Germination, %
F-6 (bulked)	50	0	25	0	25	16
F-8(3)	50	0	50	0	50	22
F-9(1)	50	0	50	0	50	4
F-12(2)	50	0	50	0	25	16
F-13 (bulked)	50	0	25	0	25	4

The difference in the treatment of the re-tested seeds and continuously stored seeds is that the former received, in the form of a germination test, a ten-day break in the continuity of dry-storage conditions. Such a short period of time removed from the after-ripening period cannot account for the observed reductions in the germination of the re-tested seeds. It was shown by special tests that seeds which were non-germinable in early tests had not suffered appreciable loss of vitality but were highly germinable (up to 76 per cent) when given an after-ripening period sufficient to overcome the state of secondary dormancy. It must be assumed, therefore, that the non-germinability of the re-tested seeds is due to a form of secondary dormancy induced by the conditions imposed by the first germination test.

Effect of Light upon Germination

The effect of light on the germination of seeds has been studied by a number of investigators. Gardner (8) found that exposure to light caused a stimulation of germination in the seeds of *Rumex crispus* and *Phoradendron flavescens* and a retardation of germination in the seeds of *Datura stramonium*. It has been reported by Morinaga (11) that light and nitrogen compounds have a favorable joint effect on the seed germination of Bermuda grass (*Cynadon dactylon*), Canada blue grass (*Poa compressa*), and cat-tail (*Typha latifolia*). Johnson *et al.* (9) found that light was necessary for the germination of some types of tobacco (*Nicotiana*) seed, but that the seeds of most of the common American varieties would germinate in the dark, although in some cases at a slower rate and with a lower percentage than when light was present.

The studies on the effect of light upon the germination of seeds of *A. fatua* were on the whole very inconclusive and, in consequence, the results are not tabulated. Both hulled and dehulled seeds were used in all tests.

A test was made using both glass and common dark germinators. During the day the glass germinator was in diffused daylight while during the night it was in practically complete darkness. In each of the four seed stocks used, the seed tested in the light gave considerably (about 15%, on the average) higher germination in the case of both hulled and dehulled seeds.

The temperature in the dark germinator during the test was approximately 20° C. while that in the glass germinator was one degree higher. Aeration may have been slightly better and humidity slightly lower in the glass germinator. It is questionable whether these differences could account for the observed differences in germination results. However, the fact that seeds with hulls intact showed increased germination in light would lead one to suspect that factors other than light were operative.

Further tests were made to determine the effects of alternate and continuous diffused light and continuous direct light upon germination. The source of continuous light was a common desk lamp with a 50-watt bulb. Direct light was obtained by placing the glass cylinder containing the seeds about 30 inches from the lamp and directly in front of it; diffused light was obtained by placing a similar cylinder the same distance to the side of the lamp out of the line of

direct illumination. The glass cylinders used in these tests were twelve inches in height and seven inches in diameter, and were well-aerated and maintained at a favorable humidity by a free-water surface. Alternate diffused light was obtained in the glass germinator as described in the previous experiment. The approximate temperatures maintained in the various tests are as follows: dark, 20° C.; alternate diffused light and continuous diffused light, 21° C.; continuous direct light, 21.5° C.

The results from this test, in contradiction to those of the preceding test, showed that in general all forms of light were detrimental, particularly continuous direct light. The fact that the seeds used in the first test were in the early stages of after-ripening while the seeds of the second test were in an advanced stage of the process suggests that light may have a stimulating effect under the former conditions and a detrimental effect under the latter conditions. Unfortunately, a lack of suitable materials prevented repetition of the experiment.

The Effect of Temperature upon Germination

A study was made of the effect of prolonged storage of wet and dry seeds at different temperatures upon subsequent germination.

After storage in the laboratory for four months, four lots of 50 seeds each were drawn from each of three different seed stocks. One lot from each seed stock was then placed under each of the following conditions: (1) continued dry storage in the laboratory; (2) moistened and maintained in a frozen state in a refrigerator held at slightly below 0° C.; (3) dry storage in an unheated seed-house vault where the temperature probably varied from a few degrees above freezing to about −18° C.; and (4) moistened and stored in moist sand in the open, shaded from the sun during the middle of the day, and experiencing temperature variations between 11° and −25° C. Storage under these various conditions extended from Dec. 6, 1934 to March 6, 1935—a 90-day period. The results of the germination tests which were begun on March 6 are summarized in Table VIII.

TABLE VIII

COMPARISON OF GERMINATION OF SEEDS STORED FOR 90 DAYS UNDER DIFFERENT CONDITIONS, NAMELY, IN HEATED LABORATORY, IN REFRIGERATOR AT 0° C. (FROZEN), IN UNHEATED SEED HOUSE, AND IN THE OPEN

Material	Percentage germination following various kinds of storage			
	Laboratory	Refrigerator	Seed house	In the open
F-12(41)	12	14	2	0
F-15(2)	18	16	4	0
F-15(5)	32	74	12	0
Average	20.7	37.7	6.0	0.0

The results indicate that the after-ripening process in dry seeds was much slower at temperatures around the freezing point (seed-house conditions) than it was at about 21° C. (laboratory conditions). Unfortunately, germination tests were not made at the time of subjecting the seeds to the different storage conditions. Such tests would have indicated the stage of after-ripening at

the beginning of the storage period, and thus would have made it possible to determine the extent to which after-ripening occurred at the different temperatures. It is possible that the after-ripening process was barely, if at all, active at the seed-house temperatures.

In the case of storage in the frozen condition (refrigerator), it does not seem reasonable to assume that the rate of after-ripening was greater than at laboratory temperatures; but, rather, it would seem quite reasonable to assume a more or less complete cessation of the after-ripening process in the frozen seeds. Therefore, an increase in germination due to storage in the frozen condition is probably best explained by assuming a relatively sudden breaking of dormancy occasioned by the effects of freezing or of thawing or of both.

The fact that the seeds stored in the open did not germinate is perhaps the result of the development of secondary dormancy through the occurrence of germinative conditions for short periods of time, such as warm days, followed by conditions, such as cold nights, which definitely checked any tendencies toward germination. (See section on secondary dormancy.) Freezing and thawing have, apparently, no permanently injurious effects upon germinability, for seeds of *A. fatua* regularly undergo these conditions in the natural environment of temperate regions.

Effect of Chemical Treatments upon Germination

Investigations were made in which the effects of the following chemicals upon the germinability of dormant seeds were tested: oxygen, ether, potassium nitrate, ethylene chlorhydrin, dichlorethylene, and sodium thiocyanate.

The effect of oxygen concentration upon germinability was tested in air-tight chambers formed by inverting two-litre Petri dish covers on special supports in a large copper pan partly filled with water. The supports were constructed in such a manner as to hold the seed containers (Syracuse watch glasses) above the water and to permit necessary manipulatory operations.

In the case of the pure oxygen test, the procedure was as follows: Syracuse watch glasses containing the seeds were filled with water to exclude the air, and each covered by a second watch glass and placed under the Petri cover. The air was then sucked from the chamber by means of a U-shaped tube until the chamber was completely filled with water. Oxygen was generated by heating a mixture of potassium chlorate and manganese dioxide, and passed into the chamber until the water was displaced to a level well below the seed containers. The watch glass covers were removed from the seed containers by wire manipulators, and the excess water sucked from the containers by a specially constructed tube.

The procedure in the case of a second set-up was essentially the same, except that only half of the air was removed from the chamber. Thus, the atmosphere in this chamber consisted of air and pure oxygen in the ratio of 1 : 1.

A third and similar set-up was used to test the effect of an atmosphere receiving one drop of ether daily, while a fourth served as an air check.

All chambers were supplied with a free liquid surface of sodium hydroxide as a precaution against the accumulation of an excess of carbon dioxide from the germinating seeds. The oxygen concentration chambers were not disturbed at any time during the test. Watering was accomplished by means of a specially designed glass tube, while germinated seeds were removed by wire manipulators. The ether and check chambers were removed and replaced daily.

The results are summarized in Table IX. Each lot consisted of 50 seeds.

TABLE IX

GERMINATION IN DIFFERENT ATMOSPHERES CONTAINED IN AIR-TIGHT GLASS CHAMBERS

Atmosphere	Germ. of F-15 (bulked), %			Germ. of F-18 (bulked), %		
	Lot 1	Lot 2	Average	Lot 1	Lot 2	Average
Air	36	32	34	4	6	5
Air : Oxygen = 1 : 1	40	38	39	26	12	19
Oxygen	32	40	36	6	2	4
Air-ether	48	52	50	0	0	0

From these data it would appear that an atmosphere of pure oxygen had little or no stimulatory effect upon germination, and that the 1 : 1 air-oxygen mixture gave slight stimulation to the F-15 seeds and considerable stimulation to the F-18 seeds. Ether, as applied in the test, appears to have stimulated germination in the F-15 seeds and to have inhibited it in the F-18 seeds.

The results from the 1 : 1 air-oxygen test are in agreement with those of Atwood (1) who obtained increased germination by subjecting *A. fatua* seeds to increased oxygen concentrations.

As a result of Morinaga's (11) report of the stimulatory effect of solutions of potassium nitrate upon the germination of the seeds of certain grasses, this chemical was tested for its effect upon *A. fatua* seeds. As a preliminary test, seeds of F-15 (bulked) were soaked for 48 hr. in potassium nitrate solutions of different concentrations as well as in tap water. The germination percentages after soaking in 0.2, 0.5, and 1.0% solutions were 70, 92, and 96, respectively, while 10% germination occurred in the water check.

The results show a very marked stimulation as a result of soaking in potassium nitrate solutions, with a consistent increase in germination as the concentration was increased from 0.2 to 1.0%.

TABLE X

PERCENTAGE GERMINATION AFTER SOAKING SEEDS IN WATER, AND IN 10 CC. OF KNO_3 OF DIFFERENT CONCENTRATIONS FOR 24- AND 48-HOUR PERIODS

Material	Water		0.5% KNO_3		1.0% KNO_3		2.0% KNO_3	
	24 hr.	48 hr.	24 hr.	48 hr.	24 hr.	48 hr.	24 hr.	48 hr.
F-6 (bulked)	0	0	2	2	20	6	64	22
F-15 (bulked)	4	0	18	8	74	18	68	56

These preliminary results suggested a further test in which the concentration of the solution was increased and the time of soaking varied. The data from such a test are given in Table X.

In all cases soaking for 24 hr. gave higher percentages of germination than soaking for 48 hr. In the case of F-6 seeds the 2% concentration gave the highest germination, while in the case of F-15 the 1% concentration was the most stimulatory.

A test was next made to determine the effect of soaking for shorter periods of time in 1% and 2% solutions, the results of which are given in Table XI.

TABLE XI

EFFECT OF TIME OF SOAKING IN 25 CC. OF 1% AND 2% SOLUTIONS OF KNO_3 UPON GERMINATION OF F-15 (BULKED)[1]

Solution	Number of hours of soaking				
	1	3	6	12	24
Water	74	—	—	—	—
1% KNO_3	78	70	72	100	98
2% KNO_3	96	96	100	100	100

[1] *The F-15 material used in this test represents a different group of bulked plants and is several weeks older than the F-15 material used in the test reported in Table X.*

It may be concluded from data given in Table XI that the process of stimulation, which is presumably related to the absorption of K^+ or NO_3^- ions by the embryo, required twelve hours to reach maximum effectiveness in the case of 1%, and six hours in the case of 2% solutions. It would appear that periods of soaking up to six hours in the 1% solution had no effect upon germination, while a one-hour soaking in the 2% solution produced a marked stimulation.

A test was next devised to determine the effect of soaking seeds for a constant length of time in different volumes of a relatively dilute solution of potassium nitrate, the results of which are summarized in Table XII.

TABLE XII

EFFECT ON PERCENTAGE GERMINATION OF SOAKING SEEDS OF F-15 (BULKED) 48 HR. IN DIFFERENT VOLUMES OF .125% SOLUTION OF KNO_3

Water	0.125% KNO_3				
	10 cc.	20 cc.	40 cc.	80 cc.	160 cc.
74	78	80	100	94	100

From these data it would appear that the stimulatory effect upon germination was increased by increasing the volume of the relatively weak solution of potassium nitrate in which the seeds were soaked. Further, this increase in stimulation appears to be similar to that obtained when the concentration of a constant volume is increased (See Table X), and to that obtained when the period of soaking is increased in a constant volume of constant concentration (See Table XI). It may be deduced from these observations that the stimulation of germinability is due to the absorption of either the K^+ or NO_3^- ion by the embryo or related tissues, the greater the number of ions absorbed up to a certain point the greater being the stimulation. The

absolute amount of ions absorbed, until the maximum is reached, is determined by the total number of ions present in the solution whether concentrated in a small volume or dispersed in a large volume, and by the amount of time which is allowed for absorption to take place.

The probability that the NO_3^- rather than the K^+ ion provides the stimulation is indicated by the fact that Morinaga (11) found that, in addition to potassium nitrate, several other nitrate compounds produced increased seed germination.

A number of other chemicals, namely, ethylene chlorhydrin ($ClCH_2CH_2OH$), dichlorethylene ($C_2H_4Cl_2$), and sodium thiocyanate (NaCNS), were tested for effect upon the germination of seeds of *A. fatua*. Denny (5) has reported each of these chemicals to be effective in forcing the sprouting of dormant potato tubers. The same author found (6) that ethylene chlorhydrin vapor also broke the rest period of freshly harvested gladiola corms. Ethylene chlorhydrin was found by Deuber (2) to be effective also in shortening the rest period of sugar maple, Norway maple, black oak, and red oak seeds.

In the present experiments, the following methods of seed treatment were used for the different chemicals:

In the cases of ethylene chlorhydrin and dichlorethylene, the seeds were immersed in 100 cc. of the solution for one minute, after which the solution was drained off and the bottle stoppered for 24 hr. When sodium thiocyanate was used, the seeds were soaked for one hour in 100 cc. of the solution, drained and placed directly upon germinator pads. The concentrations of solutions and duration of treatment used were based upon the type of treatment found most effective by previous investigators. In all cases the germinator pads were moistened thoroughly with water before receiving the seeds, and subsequently kept moist with water.

The results of these tests are given in Table XIII.

TABLE XIII

EFFECT OF VARIOUS CHEMICAL TREATMENTS UPON THE PERCENTAGE GERMINATION OF F-12 (BULKED)

H_2O, 1 hr.	$ClCH_2CH_2OH$			$C_2H_4Cl_2$				NaCNS			1% KNO_3, 24 hr.
	1%	3%	6%	0.1%	0.2%	0.5%	1.0%	1%	2%	3%	
48	0	4	0	28	24	36	0	56	50	32	96

The results of the chemical treatments, considered in relation to the results of the water check, indicate: (*i*) complete or nearly complete inhibition of germination in the case of ethylene chlorhydrin in all concentrations used; (*ii*) a complete inhibition by the 1% dichlorethylene treatment and a moderate inhibition by treatments with weaker concentrations; and (*iii*), a slight

stimulation by the 1% sodium thiocyanate treatment, with the 2% treatment indifferent and the 3% treatment moderately inhibitory. A potassium nitrate check gave almost perfect germination.

The results from the sodium thiocyanate treatments indicate the possibility of stimulation at concentrations below 1%.

General Discussion and Conclusions

The experimental results have been, with respect to the more specific points, more or less fully discussed in the sections devoted to the different experiments.

In considering at this point the implications and relations of the experimental results, it is recognized that the general and preliminary nature of the work does not permit any extensive generalization. There are, however, some points which should be discussed from the point of view of the work as a whole, particularly those concerning the causes of delayed germination and the stimulation of germination in dormant seeds.

It was shown by studies on the germinability of hybrid seeds that delayed germination is, in all probability, due to agencies operating after, and influenced by, fertilization. The results from experiments on the effect of breaking the seed coat over the embryo indicated very strongly that the seed coat (pericarp and testa) was intimately concerned with the delay in germinability. Considering these two facts together, it would appear that certain agencies affecting the seed coat during the development of the seed have a causal relation to delayed germination.

Post-fertilization agencies which occasion the absorption of several distinct tissues which envelop the ovule before fertilization are known to be operative in the oat seed (13). It may be inferred, therefore, that the property of delayed germination in *A. fatua* may be due to an inherent tendency to undergo a different type or a lesser degree of post-fertilization absorption of germination-restricting tissues enveloping the embryo than occurs in readily germinable species. A less probable inference is that delayed germination is due to an inherent capacity of post-fertilization development of tissues capable of preventing immediate germinability.

The question now arises: how do these tissues bring about delayed germination and what effect has the after-ripening process upon them? The results of the present work agree with those of Atwood (1) in indicating that an increased oxygen concentration in the atmosphere stimulates germination. Atwood also found that when seeds were subjected to increased oxygen concentrations, or when seed coats were broken, there was an absolute increase in the rate of oxygen absorption. It is universally recognized that a supply of oxygen is a prerequisite to seed germination. There appears, therefore, to be a strong probability that the tissues in question cause delayed germination by restricting the oxygen supply to the embryo. Thus, the after-

ripening process may be considered as being essentially a series of changes in the tissues enveloping the embryo which results in an increased permeability to oxygen.

The observation that germinability may be correlated with the position of the seed in the panicle, could be explained by assuming that in the case of seeds in "germinable positions", which are also positions of early maturity, the process of post-fertilization absorption of tissue is carried further than it is in the case of seeds in other positions in the panicle. This line of reasoning may also be applied in an explanation of the germinative differences between primary and secondary grains.

The whole question of post-fertilization tissue absorption or development is in a hypothetical condition; it suggests certain procedures for further investigation, especially along anatomical and microchemical lines.

Of the seed treatments used in the present work, the most efficient in overcoming dormancy were: (*i*) breaking the seed coat over the embryo, (*ii*) soaking the seeds for 12 to 24 hr. in 1% or 2% potassium nitrate solutions and (*iii*) subjecting seeds under germinating conditions to an atmosphere having an increased concentration of oxygen. The reasons for stimulation of germination in the case of coat-breaking and oxygen-concentration treatments are obvious from the above discussion. The stimulation by potassium nitrate might be considered as resulting from: (*a*) direct germinative stimulation by the absorbed anion; (*b*) indirect effects of the absorbed anion such as, for example, increased utilization of stored carbohydrates (12), or, the acceleration of organic acid formation following reduction; and (*c*) chemical effects upon the seed coat which produce increased permeability to oxygen.

Freezing of the seed also produced a certain degree of stimulation, which is to be expected on the basis of an increased permeability to oxygen through ruptures in the seed coat resulting from frost injury.

The development of secondary dormancy in partly after-ripened seeds may represent the re-establishment of a high degree of impermeability in seed coats which had nearly reached a state of permeability to oxygen.

Acknowledgments

The writer is indebted to Dr. E. F. Gaines and Dr. H. F. Clements for helpful suggestions in connection with the analysis and presentation of data, and to Mr. Lewis Weiner for excellent routine assistance.

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ASSOCIATIONS OF SOMATIC CHROMOSOMES INDUCED BY HEAT AND CHLORAL HYDRATE TREATMENTS[1]

By F. H. Peto[2]

Abstract

Germination of *Hordeum vulgare* at 35–36° C. induced, in the nuclei of the root tips, numerous fractures in one or both chromatids, particularly in the attachment region of the chromosome. The fractured ends appeared to possess an unsatisfied attraction for each other. Chiasmata were formed by the union at random of the fractured ends which happened to lie close together in the nucleus, giving rise to associations of one and one-half, two, and three chromosomes involving chromatid interchange or crossing-over between non-homologous as well as homologous chromosomes. The manner in which fragmentation, translocation, and elimination of chromosome parts can occur in somatic tissue is demonstrated. Somatic segregation and the incidence of new linkage relationships are discussed.

The formation of tetraploid and octaploid nuclei was induced in root tips of *Pisum sativum* by treatment with chloral hydrate. The chromosomes of these polyploid nuclei were frequently closely associated in pairs which bore a superficial resemblance to the paired associations observed in barley. The chromosomes, however, were never united by chiasmata and retained their juxtaposition solely through their inability to separate normally at anaphase.

Introduction

Pairing of chromosomes in somatic divisions occurs in a large number of plant and animal species. Watkins (19) lists 32 species of plants in which this phenomenon is known to occur and Metz (13, 14) reports somatic pairing of homologues in approximately 80 species of *Diptera*. In the majority of cases this pairing is simply a side-by-side association of homologues or daughter chromosomes without any evidence of their being united by chiasmata. In many instances this juxtaposition is maintained by virtue of homologous attraction as is the case in *Diptera*. In others the paired relationship is initiated by faulty separation of the daughter chromosomes after equational division.

In meiosis of most plants and animals the paired condition of the homologues is maintained between diplotene and the beginning of anaphase by means of chiasmata. A chiasma is defined as an exchange of partners in a system of paired chromatids (Janssens, 7). Chiasmata have never been observed in somatic pairing with the exception of an isolated case reported by Kaufmann (8) in *Drosophila melanogaster*. The present work includes a study of somatic pairing induced by heat treatment of barley and a re-examination of the somatic pairing obtained by Kemp (9) in peas (*Pisum sativum*) treated with chloral hydrate.

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Contribution from the Division of Biology and Agriculture, National Research Laboratories. Ottawa.

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Methods and Germination Data

The technique employed to induce the association of chromosomes in the root tips of O.A.C. 21 barley varied slightly in the four treatments reported. All the treatments, however, involved the germination of the seeds at or near the critical temperature of 35–36° C. The seminal root tips were fixed in La-Cour's 2 B.E., sectioned 13 μ thick, and stained with gentian violet (La-Cour, 11).

In Treatment 1 the apparatus used for germinating the seeds consisted of a constant temperature water bath in which half pint sealers containing the seeds were immersed for the treatment period. The seeds were placed on a layer of sand which was saturated with nutrient solution. Preheated air was drawn continuously through the sealer to provide adequate ventilation. As a precaution against the growth of bacteria and molds all the equipment was steam sterilized and the seed was sterilized for five minutes in a solution of 0.5% mercuric chloride and subsequently washed thoroughly in distilled water. The air supply was also sterilized by bubbling it through a 2% solution of hydrochloric acid containing 5% mercuric chloride. The temperature of the bath was maintained at 35° C. and the treatment lasted for 96 hours. At the end of this period 38% of the seeds had produced seminal root tips 4 to 12 mm. long.

The same apparatus and germinating temperature were used for Treatment 2, but the seed was not sterilized and cotton batting was used in place of sand. After 42 hours 66% of the seeds had germinated and root tips were collected from 25 seedlings.

In Treatment 3 an attempt was made to stimulate germination under high temperature conditions by bubbling oxygen through the jars. The treatment and results are given in Table I. It was found that the oxygen apparently did stimulate germination to some extent. The treatment temperature varied between 35.5 and 36.0° C.

TABLE I

EFFECT OF OXYGEN ON GERMINATION IN TREATMENT 3

Treatment	Duration of treatment, hr.	Germination, %
Increased oxygen	26	54
Normal aeration	26	46
Increased oxygen	36	59
Normal aeration	36	31

A Hearson germinator was used instead of a water bath in Treatment 4. The seed was placed on absorbent cotton in crystallizing dishes and the absorbent cotton was kept damp with nutrient solution. Four lots of 100 seeds each were germinated for 41 hours at 35.5–36° C. They were then removed from the germinator and allowed to grow at room temperature. Root tips were fixed at 0, 12, 24 and 48 hours after removal from the germinator. The percentage germination for the four lots was 57, 55, 37 and 28. This wide variation was thought to be the result of the space variation of the

temperature in the germinator. It was extremely difficult to duplicate germination results at these critical temperatures since the slightest temperature variation caused very wide fluctuations in germination percentage.

The method employed in the treatment of *Pisum sativum* with chloral hydrate was similar to that used by Kemp (9). Arthur peas were germinated in a Hearson germinator at 18° C. and after four days, 90% of them had germinated. One hundred and twenty uniform seedlings were selected and grown in nutrient solution until they had attained an average height of $2\frac{1}{2}$ inches. The roots of 35 of these plants were then washed carefully to remove the nutrient solution, and immersed in a 0.75% solution of chloral hydrate for one hour. After treatment the roots were again washed in six changes of water for one hour before being replaced in the nutrient solution. A single root tip was taken from each seedling 0, 24 and 72 hours after treatment and fixed for cytological examination.

The plants grew vigorously after being removed from the chloral hydrate and no external indications of permanent injury were noticeable.

Chromosome Associations in Root Tips of Heat Treated Barley

Cytological preparations of root tips from 20 seedlings of Treatment 1 were examined. The high temperature had checked growth to such an extent that division figures were found in root tips of only nine of these seedlings. The division figures were relatively normal in so far as there was no clumping of the chromatin or other definite evidences of degeneration such as observed after treatment with chloral hydrate. A complete analysis was made on all the division figures observed and the resulting data are shown in Table II.

Twenty-five per cent of the nuclei contained one or more of the abnormalities indicated in the table. In addition to associations of two chromosomes and fragments there was a large number of tetraploid nuclei. The latter seemed to be scattered throughout the meristematic region of the root tip and large tetraploid sectors were never observed. This suggests that they had originated in recent cell divisions and this opinion is confirmed by the juxtaposition of what are obviously daughter chromosomes in these tetraploid cells (Fig. 23). It is believed that the tetraploid cells arose through failure of the chromosomes to separate after splitting, and consequently the daughter chromosomes have maintained their juxtaposition. This paired condition would hardly be maintained in subsequent divisions.

In Treatment 2, 66% of the seed germinated, which was 28% more than in Treatment 1, and, as might be expected, there were fewer abnormalities. Similar results were obtained in Treatment 3 except that a larger number of tetraploid nuclei were observed. The number of nuclei analyzed in this treatment was too small to show any differences between the lots which had been germinated under normal aeration and with increased oxygen concentration.

TABLE II

COMPLETE ANALYSIS OF METAPHASE NUCLEI IN HEAT TREATED BARLEY

Treatment	Fixation	Nuclei analyzed	Normal nuclei		Chromosome association			Fragments			Chromosomes with one broken chromatid	Tetraploid nuclei
			No.	%	Two	One and one-half	Three	Broken at attachment constriction	Small	Large		
1	Immediately after treatment	144	108	75.0	13	0	0	7	1	1	1	20
2	Immediately after treatment	93	85	91.4	4	0	0	2	1	0	0	1
3	Immediately after treatment	60	42	70.0	8	1	0	1	0	0	0	9
4	Immediately after treatment	0										
4	8 hr. after treatment	233	148	63.5	33	8	1	210	5	2	4	1
4	24 hr. after treatment	141	112	79.4	13	8	1	146	2	0	3	0
4	48 hr. after treatment	114	110	96.5	0	0	0	2	5	0	0	0
Totals		785	605	—	71	17	2	368	14	3	8	31

The object of Treatment 4 was to ascertain the frequency of the cytological abnormalities after various normal growth periods subsequent to the completion of the treatment. The treatment was so severe that there were no division figures in the root tips taken immediately on removal from the treatment. Eight hours after treatment the division figures were again very abundant, and 36.5% of the nuclei analyzed contained various chromosomal abnormalities in the proportions recorded in Table II. Twenty-four hours after treatment 20.6% of the nuclei analyzed were abnormal, while after 48 hours only 3.5% were abnormal. This rapid disappearance of these abnormalities is in accord with the observations on the disappearance of chromosomal abnormalities in seedlings grown from aged and heat treated seed (16).

The mode of origin of the paired associations can be deduced from observations at prophase and metaphase stages, as well as from the occurrence in the same nucleus of fragments, chromosomes with broken chromatids, and associations of $1\frac{1}{2}$ chromosomes. The majority of the pairs have a chiasma located at or near the attachment constriction (Figs. 2, 9, 10, 17 and 21). This suggests that the breaks and re-attachments of the chromatids to form these chiasmata took place in the attachment constriction region. This conclusion is substantiated by the observation that chromatid fracture occurred over twenty times more frequently in this region of the chromosome than in any other. In Treatment 4, examined eight hours after treatment, there were 210 fragments of this kind in 233 nuclei. Furthermore, these fragments were often found in the same nucleus with associations of two and three chromosomes (Figs. 3 and 7). Chromosomes with one broken chromatid were also observed at prophase and metaphase as shown in Figs. 24, 25 and 26.

It was not possible to determine the exact time of occurrence of the chromatid fractures, but it must have been subsequent to the splitting of the chromosome and prior to the prophase stage at which fractures have been observed. Typical examples of the earliest stages at which pairs have been observed are shown in Figs. 27 to 30. It will be noted in Fig. 29 that two of the chromatid ends are not yet united. Whether or not the broken ends of chromatids can be reunited at any stage of nuclear division has not yet been determined.

The formation of the paired associations can be explained as follows: The high-temperature growth condition causes numerous chromatid breaks to occur, mainly in the attachment regions. The fractured ends of the chromatids evidently possess an unsatisfied attraction or bond and will unite with the fractured ends of any other chromatids lying in the vicinity. Huskins and Hunter (4) came to a similar conclusion from observations on X-rayed microspores of *Trillium erectum*. If this unsatisfied attraction did not exist it would be expected that the broken ends might also unite with the ends of normal chromosomes, but configurations which would indicate that this had

taken place were never observed. When two chromosomes, each with one broken chromatid in the attachment region, lie close together in prophase, they would probably unite to form one of the types of paired associations observed.

The paired associations were classified in an attempt to determine whether the re-attachment of the broken chromatids was at random or whether there was any special attraction between the broken ends of chromatids of homologues fractured in the same region. Unfortunately it was impossible to identify positively all the chromosomes. The heteromorphic class (Table III) contains all the pairs in which the chromosomes are obviously non-homologous or which have united to form a configuration with unequal arms. Examples of heteromorphic pairs are shown in Figs. 3, 7 and 17. The last named figure is positive proof of non-homology since one of the chromosomes with a trabant was found unpaired and the other was included in a pair. The same kind of proof is shown in the association of three in Fig. 4, and the association of $1\frac{1}{2}$ in Fig. 18. The occurrence of associations of three (Figs. 3 and 4), regardless of their morphological peculiarities, is in itself a proof of the union of broken ends of non-homologous chromatids, since there are only two homologous chromosomes in this diploid species. If heteromorphic pairs occur in the primordia of the spike, a further proof of non-homologous crossing-over should be found in the heterotypic division where rings of four chromosomes should occasionally be observed. McClintock (12) uses a similar proof of segmental interchange in maize.

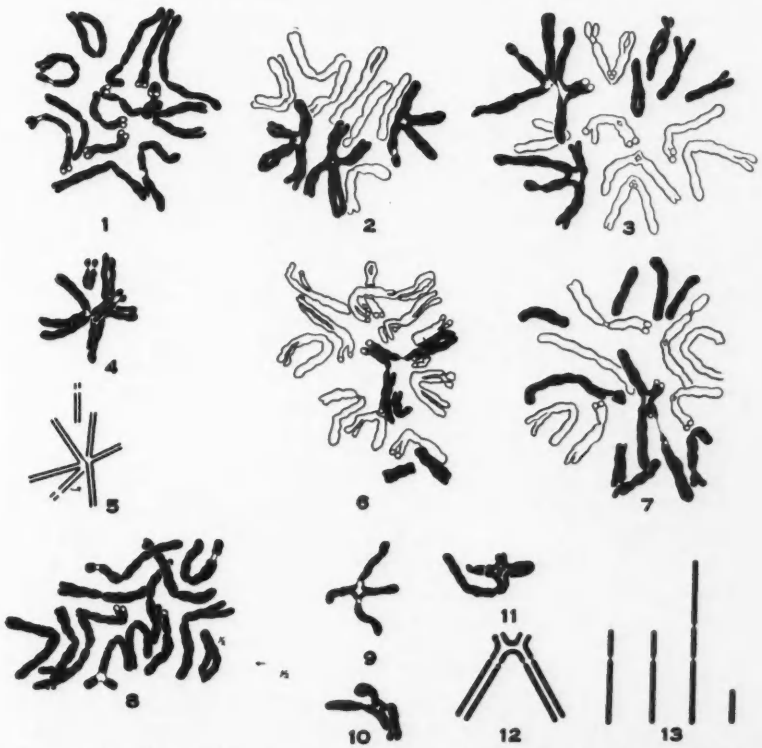
The homomorphic class in Table III includes associations of similar appearing chromosomes which may or may not be homologous. This class was sub-divided according to the way in which the fractured ends were united to form chiasmata. The normal type as shown in Figs. 9 and 10, and Plate I, Fig. A, is the result of the union of fractured ends to form a chiasma similar to that formed at meiosis. The abnormal type of homomorphic bivalent is shown in Figs. 11 to 16. The identification of this type was most positive

TABLE III

TYPES OF PAIRED ASSOCIATIONS AT METAPHASE IN HEAT-TREATED BARLEY

Treatment	Fixation	Homomorphic		Heteromorphic	Unclassifiable	Total
		Abnormal	Normal			
1	Immediately after treatment	3	4	2	5	14
2	Immediately after treatment	0	0	2	2	4
3	Immediately after treatment	4	0	1	3	8
4	Immediately after treatment	0	0	0	0	0
4	8 hr. after treatment	13	9	10	17	49
4	24 hr. after treatment	1	3	7	7	18
4	48 hr. after treatment	0	0	0	0	0
Totals		21	16	22	34	93

in pairs with subterminal chiasmata. Instead of the diagonal ends of the broken chromatids uniting as must be the case in normal meiosis, the adjacent homologous or homomorphic ends united. This is evidently what happened in the configuration shown in Fig. 11. The interpretation of the chromatid arrangement is shown in Fig. 12, and the expected constitution of the daughter chromosomes is shown in Fig. 13. One of the daughter chromosomes would be a small fragment without any attachment constriction and the halves of this fragment would likely be homologous. The long daughter chromosome would have two attachment constrictions and again the halves would likely

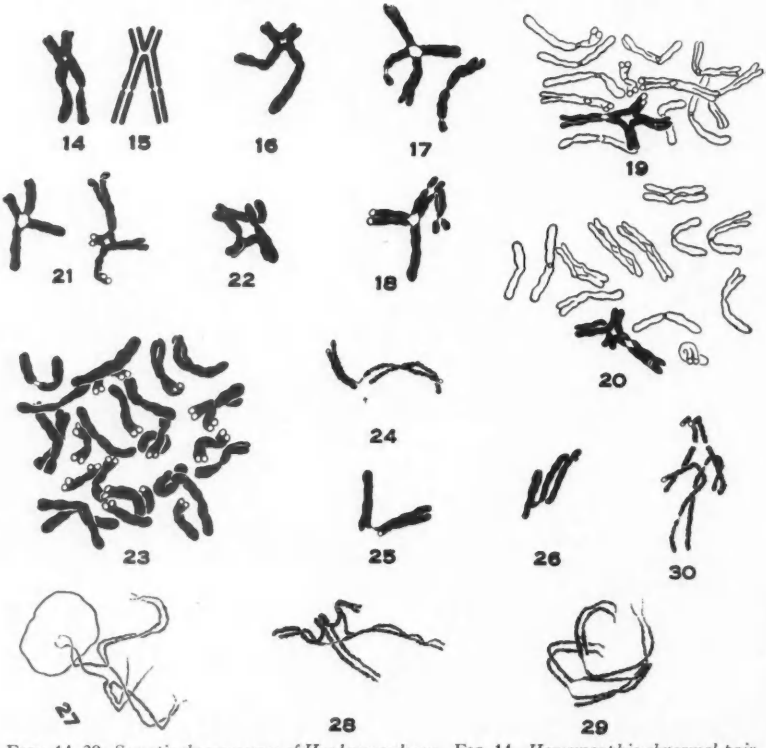


FIGS. 1–13. *Somatic chromosomes of Hordeum vulgare.* FIG. 1. *Normal, untreated.* FIG. 2. *Three pairs of chromosomes.* FIG. 3. *Associations of two and three, four half chromosomes.* FIG. 4. *Chromosome with trabant included in association of three; half chromosome with trabant unattached.* FIG. 5. *Diagram of Fig. 4.* FIG. 6. *Associations of one and one-half, three half chromosomes.* FIG. 7. *One pair with broken chromatid, one chromosome with broken chromatid, one chromosome with abnormally short arm, six half chromosomes.* FIG. 8. *Double trabant, two half chromosomes.* FIG. 9. *Homomorphic normal pair.* FIG. 10. *Homomorphic normal pair.* FIG. 11. *Homomorphic abnormal pair.* FIG. 12. *Diagram of Fig. 11.* FIG. 13. *Diagram of the separate chromatids of Fig. 11.*

Magnifications: Figs. 1, 10, 19, 20, 21, 23, × 2250. Figs. 11, 14, 16, × 2750. Remainder × 2400.

be homologous. It would be deficient, however, by the region included in the small chromosome. The two other daughter chromosomes would be normal. The above case is an excellent illustration of how duplications and deficiencies can arise in somatic tissue.

The data resulting from a classification of the paired associations are given in Table III. There is a total of 37 homomorphic, 22 heteromorphic and 34 unclassifiable pairs. If homologous chromosomes could have been accurately detected and the union of broken chromatids was purely at random, there should be six times as many heteromorphic as homomorphic pairs. However, the similarity of many of the chromosomes in the complement may have resulted in many of the non-homologous pairs being classified as homomorphic.



FIGS. 14–29. *Somatic chromosomes of Hordeum vulgare.* FIG. 14. *Homomorphic abnormal pair.* FIG. 15. *Diagram of Fig. 14.* FIG. 16. *Homomorphic abnormal pair.* FIG. 17. *Heteromorphic pair plus chromosome homologous to one included in pair.* FIG. 18. *Association of one and one-half plus homologous half chromosome.* FIG. 19. *Unclassifiable pair.* FIG. 20. *Unclassifiable pair.* FIG. 21. *Two unclassifiable pairs.* FIG. 22. *Pair at early anaphase.* FIG. 23. *Tetraploid nucleus.* FIG. 24. *Prophase chromosome with broken chromatid.* FIG. 25. *Metaphase chromosome with broken chromatid.* FIG. 26. *Metaphase chromosome with broken chromatid.* FIG. 27. *Pair at prophase, outline of nucleolus.* FIG. 28. *Pair at prophase.* FIG. 29. *Pair at prophase with two unattached ends of chromatids.* FIG. 30. *Pair at prophase. Magnifications: Figs. 19, 20, 21 23, 30, × 2250. Figs. 14, 16, × 2750. Remainder ×2400.*

This would reduce the proportion of heteromorphic pairs. In spite of this difficulty of classification the positive examples combined with the general data make it apparent that pairing of the chromosomes is not the result of any specific attraction between homologues but rather a haphazard or random union of broken chromatids. This is further emphasized by the observed proportion of 16 normal to 21 abnormal homomorphic pairs and also by the fact that homomorphic or homologous chromosomes do not show any tendency to pair unless they are united by chiasmata.

Associations of one and one-half chromosomes are shown in Figs. 6 and 18. These probably were produced either through the union of a chromosome possessing one broken chromatid with a half chromosome, or through the attachment of the broken chromatids of three half chromosomes. These associations occurred with about $\frac{1}{4}$ of the frequency of the paired condition.

Chromatid breaks are not confined to the attachment constriction region as shown by the double trabant in Fig. 8, the broken chromatid in the sub-terminal region of one of the chromosomes in Fig. 7 and the sub-terminal chiasmata in Figs. 11, 14 and 16. The possibility should also be considered that many more breaks than are shown at metaphase may occur throughout the length of the chromosome and may be covered up by the immediate reunion of broken chromatids. There is also great likelihood of many of the halves of non-homologous chromosomes being united to form normal appearing chromosomes. The reuniting of chromosome halves, whether they are homologous or non-homologous, may account for the absence of fractured chromosomes 48 hours after treatment.

A number of different re-arrangements of chromosome segments have been found in maize (Rhoades and McClintock, 17). These were believed to have resulted from a two-by-two union of broken ends of chromosomes. The broken ends of barley chromosomes may also unite in this manner but the evidence shows that frequently only one of the two chromatids at a particular locus breaks, so that the observed chiasmata are formed on re-attachment with the broken ends of other chromatids. It seems likely that some of the new chromosomal types in maize may have originated in this manner rather than by the method postulated by Rhoades and McClintock.

Chromosome Pairing in Polyploid Nuclei of Peas

The data on the analysis of the metaphase nuclei in root tips of peas treated with chloral hydrate are shown in Table IV. Those specimens that were fixed immediately after treatment with chloral hydrate were very abnormal. Nuclear division was almost completely checked and only in a few root tips was it possible to obtain chromosome counts. In the majority of the cells there was a marked coagulation of the nuclear material so that it often appeared as an amorphous mass. In those in which counts were possible, the chromosomes at metaphase appeared to be shorter and thicker than usual and their margins were abnormally rough. All the nuclei analyzed had nevertheless the normal complement of 14 chromosomes.

TABLE IV

ANALYSIS OF METAPHASE NUCLEI IN PEAS TREATED WITH CHLORAL HYDRATE

Treatment	Fixation	Nuclei analyzed	Normal nuclei	Tetraploid nuclei	Octaploid nuclei
5	Immediately after treatment	25	25		
5	24 hr. after treatment	59	35	19	4
5	72 hr. after treatment	33	13	20	0

After 24 hours' growth subsequent to treatment, an unusually high proportion of the nuclei were in active division and there was no evidence of nuclear coagulation so apparent in the material examined immediately after treatment. A normal nucleus containing 14 chromosomes is shown in Plate I, Fig. F. Polyploid nuclei were very frequent in these root tips, 19 tetraploid and 4 octaploid nuclei being observed. One diploid nucleus was found with two fractured chromosomes, but none of the other abnormalities found in the heat-treated barley were seen. Only a limited number of the apparently normal nuclei were examined.

The root tips examined 72 hours after treatment were very similar to those examined after 24 hours. Twenty tetraploid but no octaploid nuclei were found. Many apparently normal nuclei were observed, but here again only a limited number were analyzed.

Koshuchow (10) found numerous tetraploid areas in the root tips of *Cucumis sativus* L. and *Zea Mays* var. *indenta* which had been subjected to heat and cold treatments. These cells were found only in the oldest part of the periblem and never in the neighborhood of the initial cells, the endodermis, the pericycle or the central cylinder. In the root tips of peas treated with chloral hydrate, the polyploid nuclei were scattered throughout the various tissues of the meristematic region. Large polyploid sections were never observed, but it would not be expected that many divisions could have occurred within 72 hours after treatment.

Excellent evidence was available on the manner in which polyploid nuclei were formed. In many instances division and separation of the chromosomes to the poles appears to proceed normally, but cytokinesis is apparently checked and no dividing wall is laid down. An example of this is shown in Plate I, Fig. C, in which there is a cell containing two nuclei, each at the metaphase stage. If a restitution nucleus is not formed immediately, these chromosomes may split again, resulting in a tetrapolar spindle of which an example is shown in Figs. 33 and 34. This cell contains four nuclei, each with 14 chromosomes. Nemec (15) found multipolar spindles in giant cells of galls caused by *Heterodera radicicola* on the roots of sugar beets. He believed that these multipolar spindles resulted in a reduction in the chromosome number in polyploid nuclei and that this accounted for their disappearance in subsequent

PLATE I

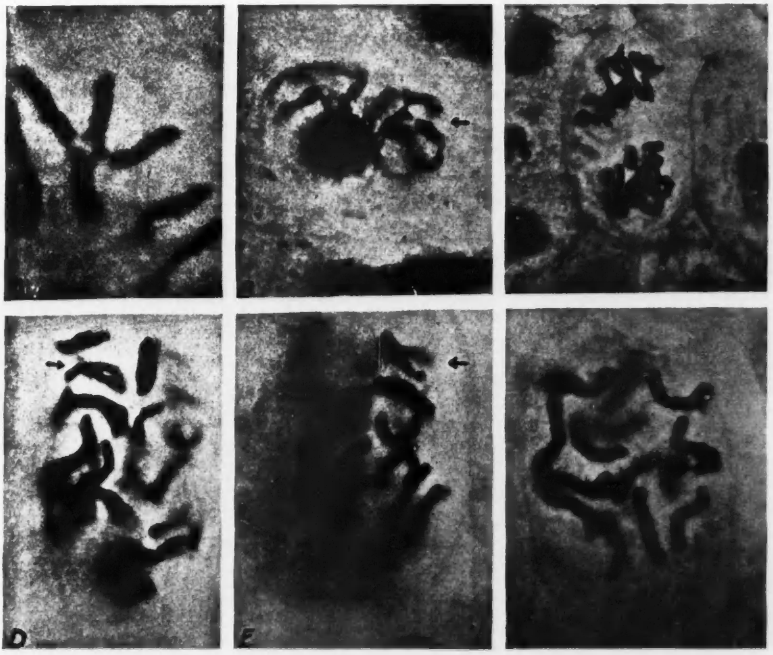
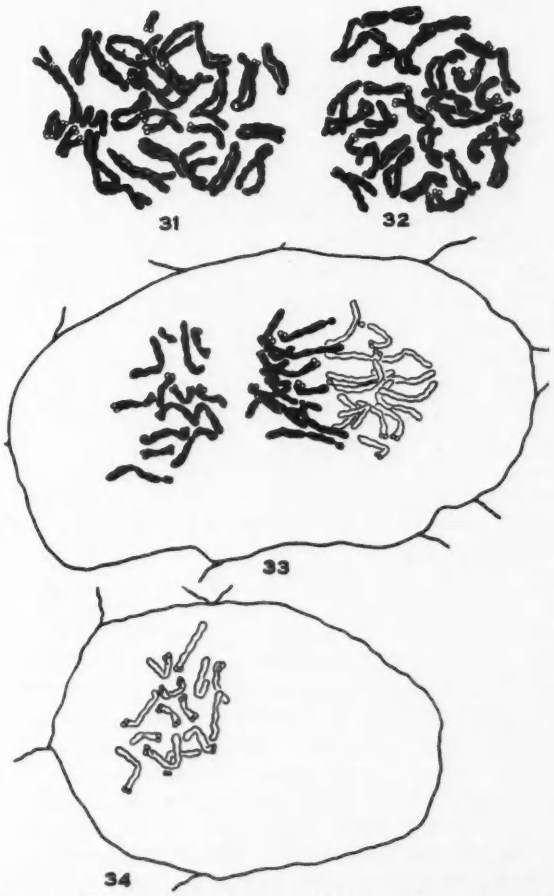


FIG. A. *Somatic association of two chromosomes in Hordeum vulgare.* ×*5000.* FIG. B. *Prophase of mitosis in Hordeum vulgare showing a partial chiasma.* FIG. C. *Two nuclei at metaphase in the same cell of Pisum sativum.* ×*1500.* FIG. D. *Octaploid cell in Pisum sativum showing chromosome pairs without chiasmata.* ×*3250.* FIG. E. *Focus at different level in same cell as shown in Fig. D.* ×*3250.* FIG. F. *Normal nucleus of Pisum sativum.* ×*3250.*

divisions. Nemec's findings are in contrast to the interpretation of the multipolar spindles found in *Pisum sativum* which in the absence of normal cytokinesis are believed to give rise to the polyploid nuclei. It is suggested that the latter interpretation may be the true explanation of the origin of the multinucleate condition in the galls of the sugar beet. This would account for the occurrence of polyploid cells if restitution nuclei were formed, but not for their disappearance.



FIGS. 31–34. *Somatic chromosomes of Pisum sativum.* FIGS. 31 AND 32. *Octaploid nuclei showing association of the chromosomes in pairs without chiasmata.* FIG. 33. *Portion of giant cell containing three groups of 14 chromosomes.* FIG. 34. *Remaining portion of the above cell containing the fourth group of 14 chromosomes. Magnifications:* × *2400.*

It frequently happens that there is a faulty separation of the daughter chromosomes at anaphase. In these cases the chromosomes remain in a paired arrangement as shown in the octaploid nuclei in Figs. 31 and 32, and Plate I, Figs. D and E. This is undoubtedly the type of pairing reported by Kemp (9) in peas, and Huskins and Smith (5) in sorghum. It is apparently not due to any attraction between homologues, but rather to a failure of the spindle mechanism. There is a further argument against this paired arrangement in peas being due to homologous attraction in that it is never observed in diploid nuclei, nor are associations of four and eight chromosomes observed in tetraploid and octaploid nuclei. Pairing of homologous chromosomes in somatic diploid nuclei has, however, been reported by Gates (2) in *Oenothera*, by Janaki-Ammal (6) in *Nicandra physaloides* and by Watkins (19) in Yucca as well as many instances in *Diptera* by Metz (13, 14) and others.

It is readily understandable how some of the early workers who observed the type of paired arrangement found in the polyploid cells of peas should suggest its relationship to normal meiotic pairing. The importance of chiasmata in retaining the paired association from diplotene to metaphase of meiosis was not appreciated at that time. Critical examination of the somatic pairs in *Pisum sativum* which appear superficially much like true bivalents (Plate I, Figs. D and E) reveals that chiasmata are not present. This evidence is presented to show especially the contrast between the paired association without chiasmata found in the peas treated with chloral hydrate and the paired association involving chiasmata found in the heat-treated barley.

Discussion

The chromosome associations found in the root tips of the heat-treated barley clearly demonstrate how chromatid interchange or crossing-over can be accomplished in somatic tissue. There can be no doubt that the chiasmata observed in this material are the result of breaks and reattachments of chromatids and that the presence of the chiasmata is substantial proof that chromatid interchange has taken place. The homomorphic abnormal pairs shown in Figs. 11, 14 and 16 illustrate this point. The abnormally short chromatids lacking attachment constrictions and the abnormally long chromatids with two attachment constrictions could hardly have arisen in any other manner than by chiasma formation involving chromatid break and reattachment.

Mottling, unstable translocations, and duplications in *Drosophila melanogaster* have been recently explained by Stern (18) on the basis of somatic crossing-over and segregation. The mechanism by means of which this crossing-over and segregation could be accomplished in somatic tissue has never been satisfactorily demonstrated. The only known instance of chiasma formation in somatic tissue was reported by Kaufmann (8) in the ganglia cells of larvae of *Drosophila melanogaster*. He found that the four chromatids of the tightly appressed homologues opened out into two planes giving distinct chiasma-like configurations, and that the pairing of the chromosomes

reached its maximum expression during late prophase. These paired chromosomes began to separate at this stage and at metaphase they were not in contact. Kaufmann did not consider that the presence of these chiasmata was sufficient proof that crossing-over had taken place. His observations, therefore, do not give a satisfactory explanation of somatic segregation and crossing-over.

The elimination or duplication of a part of a chromosome could result in sectorial effects similar to that expected from somatic segregation. Both deficiencies and duplications are involved in the chromatids of the homomorphic abnormal pairs shown in Figs. 11, 14 and 16. If in anaphase separation of the chromatids in Fig. 11, the longest chromatid with the two attachment constrictions were included in one daughter nucleus and the remaining three chromatids in the other, then the nucleus containing the abnormally long chromosome would be deficient by two short homologous portions. The missing portions would constitute the short chromosome lacking the attachment constriction. If such a nucleus were viable it would likely produce an altered character expression which might be interpreted as being the result of gene mutation or somatic segregation. These possibilities are pointed out to indicate the difficulties in determining the cause of sectorial chimeras which superficially appear to be the result of somatic segregation. Such a case was reported in the Bartlett pear by Gardner, Crist and Gibson (1).

Horlacher and Killough (3) observed cotton bolls giving 100% normal leaf progeny on plants grown from heterozygous seed which had been subjected to X-ray treatment. One of their two possible explanations of this phenomenon was that the chromosome carrying the recessive gene was deleted by the X-ray treatment and that all the functional gametes possessed only the gene for normal leaf. The observations on the heat-treated barley suggest one mechanism by which deletions of parts of chromosomes could occur.

Chiasma formation in somatic bivalents of barley differ from those found by Kaufmann in *Drosophila* in that the homologues of the latter are closely paired in all cases and chiasmata always unite homologous chromosomes. In the heat-treated barley there is no evident attraction between homologous chromosomes. The chromatid breaks apparently leave the fractured ends with an unsatisfied attraction which results in their being united at random with any others located in the vicinity. Under these conditions chromatid interchange between non-homologous chromosomes and the consequent formation of new linkage relationships would be of frequent occurrence. Heat treatments may therefore be useful in breaking the linkage between desirable and undesirable plant characters or in the formation of a new linkage of genes for desirable characters. However, it is not yet known whether chromatid interchange can be induced by heat treatments in species other than *Hordeum vulgare.*

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THE BREEDING OF DISEASE-RESISTANT SMOOTH-AWNED VARIETIES OF BARLEY[1]

By W. H. Johnston[2] and O. S. Aamodt[3]

Abstract

Barbing of awn, earliness of heading, height of plant and disease reaction were investigated in reciprocal crosses between Glabron and Trebi. Barbing of awn was also studied in reciprocal crosses between Velvet and Trebi. Rough-, intermediate- and smooth-awned plants were obtained in the ratio of 12:3:1 in the F_2. F_3 studies confirmed the F_2 findings that two factors, explained on the basis of epistasis, governed barbing of awns.

The inheritance of the characters, earliness of heading and height of plant could best be explained by assuming polymeric factors. Both these characters were greatly influenced by the environment.

In the genetic studies of stripe reaction Trebi proved highly resistant, and Glabron moderately resistant, while the progeny of the cross between them generally resembled the Trebi parent. No evidence of transgressive reaction for greater resistance was shown. The results indicate that the method of floral inoculation used was not altogether satisfactory.

A small positive correlation was found between percentage stripe infection and mean height of plant, whereas no significant correlation was disclosed between stripe reaction and either mean number of days from emergence to heading or barbing of awns. A high degree of association was found between mean height of plant and mean number of days from emergence to heading.

The status of the loose smut disease of barley in relation to the breeding of new resistant smooth-awned varieties is discussed.

Introduction

When a character which may assume the role of a limiting factor is overlooked in a breeding project, the resulting improved variety is likely at any time to become a failure as far as the producer is concerned. This principle is particularly applicable to the problem of breeding for disease resistance in field crop work. In the breeding of smooth-awned barleys it is important that such a desirable character as smooth awn be associated with the equally desirable one of disease resistance.

Certain progress has already been made in this direction. The Minnesota Agricultural Experiment Station has introduced several smooth-awned varieties resistant to the spot blotch disease (*Helminthosporium sativum*) (19, 20). The Wisconsin Agricultural Experiment Station has been concentrating on the development of new smooth-awned varieties possessing resistance to the stripe disease, *Helminthosporium gramineum* (38). Griffee (12) studied the reaction of barley hybrids to *H. sativum* in crosses between rough- and smooth-awned parents. Isenbeck (21) investigated stripe reaction in crosses involving Velvet as one parent. Recently Powers and Hines (31) studied the resistance to three forms of stem rust in barley crosses between Peatland (rough-awned,

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resistant) and Glabron (smooth-awned, susceptible). They concluded that no difficulty should be encountered in obtaining smooth-awned selections, resistant to the forms of rust used in their investigation.

In 1929, a barley breeding project was begun at the University of Alberta, Edmonton, for the purpose of developing new, smooth-awned, disease-resistant, barley varieties suitable for Alberta conditions. Attention was confined mainly to covered smut and stripe, although some attention was given to loose smut. During the course of this investigation genetic data were also compiled on barbing of awn, earliness of heading and height of plant. The results obtained regarding the covered smut phase of this investigation have been reported by Johnston (23), and Aamodt and Johnston (2).

Materials and Methods

The barley varieties used as parents were two smooth-awned types, Glabron and Velvet, and a rough-awned type, Trebi. Reciprocal crosses were made between Trebi and each of the smooth-awned varieties.

Trebi is a six-rowed, rough-awned variety originating as a pure line selection in 1907 in the co-operative breeding experiments conducted by the United States Department of Agriculture and the Minnesota Agricultural Experiment Station at St. Paul (17). Trebi is mid-late in maturity, mid-tall, susceptible to *Helminthosporium sativum*, moderately susceptible to *Ustilago hordei* and resistant to *Ustilago nuda* and *Helminthosporium gramineum*.

Glabron and Velvet are six-rowed, smooth-awned varieties developed at the Minnesota Agricultural Experiment Station (19). Glabron was developed from a backcross between Smooth Awn (a selection from a cross between Lion and Manchuria) and Manchuria, whereas Velvet resulted from a cross between Smooth Awn and Luth. Both Glabron and Velvet possess a good length of straw and are mid-late in maturity. A summary of the agronomic performance of these two varieties in Alberta for the five year period 1930-34 has recently been made by Aamodt and Johnston (1). These varieties are resistant to *U. hordei* and *H. sativum* and susceptible to *U. nuda*. Velvet is highly susceptible to *H. gramineum*, whereas Glabron is moderately so.

The crosses involving Trebi and Velvet were used only to study the inheritance of barbing of awn in the F_2. This character was also studied in the F_1, F_2 and F_3 of crosses between Glabron and Trebi. Earliness of heading, height of plant, and disease reaction were studied in the F_3 of the last-named cross. The F_3 lines were grown in ten-foot rows with approximately 50 seeds per row. Parental check rows were sown at frequent intervals.

Barbing of Awns

The results of the more important studies on the inheritance of barbing of awns are given in Table I. There is a considerable difference in the mode of inheritance of barbing of awns reported. Since different parental material was used by the various investigators, the differences in results obtained and the conclusions drawn are not unexpected.

TABLE I

SUMMARY OF RESULTS OF STUDIES BY A NUMBER OF INVESTIGATORS ON THE INHERITANCE OF BARBING OF AWN IN BARLEY

Investigator	Materials	Behavior
Harlan (13)	Several crosses	Rough awn dominant, single factor.
Vavilov (50)	Several crosses	5–6 pairs of factors.
Hayes *et al.* (20)	Lion × Manchuria	One main factor, plus modifiers.
Griffee (12)	Lion × Svanhals	Two factors explained on basis of epistasis.
Sigfusson (39)	Bearer × Lion Chinese × Lion	Two complementary factors.
David (7)	Glabron × Trebi Velvet × Trebi	One main factor for smooth awn and one main factor inhibiting smooth awn.
Robertson, Deming and Koonce (33)	Coast × Lion	Two factors explained on basis of epistasis.
Wexelsen (52)	Machine × Smooth Awn 4252	Single factor.

Methods and Experimental Results

The degree of barbing of the awns was estimated simply by passing the awns between the fingers. This simple and rapid method was adopted rather than the more complex index system, because of the large number of individuals in the F_2 population to be studied and because it was thought that, in the event of using an arbitrary index, the true phenotypic classes might be overlooked. An examination of a large number of plants of the smooth-awned parents showed a considerable variation in the degree of barbing of the awns. This varied from a few barbs at the extreme tips of the awns to barbs extending one-quarter of the length of the awns.

The F_1 of all the crossses studied were rough-awned. It was a comparatively simple matter to separate the F_2 plants into two main groups: those with completely rough awns and those with awns exhibiting some degree of smoothness. Greater diffiiculty was experienced in establishing classes within these two groups. After considerable study the following awn classes were decided upon:

1. Rough } rough
2. Near-rough }
3. Smooth-base } intermediate
4. Near-smooth }
5. Smooth smooth

The first two classes have fully barbed awns, but the barbs of the second class are much less scabrous than those of the first. The awns of Class 3 have prominent barbs extending along 50–75% of their length, whereas in Class 4 the barbs seldom extend over 50% of the length of the awn and are

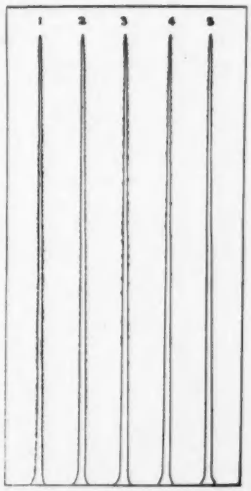

FIG. 1. *Diagrammatic length-wise section of average awn of each of five awn classes: (1) rough, (2) near-rough, (3) smooth-base, (4) near-smooth, and (5) smooth; used in classification of F_2 of reciprocal crosses of Glabron and Trebi and Velvet and Trebi.*

not as scabrous as those of Class 3. Class 5 reflects the smooth awn of the parental type. An average awn of each of the five classes is illustrated diagrammatically in Fig. 1.

Considerable variation was observed within the two rough-awned and two intermediate classes. Since environmental factors might be responsible for these variations, the segregation of F_2 plants for barbing of awn in the crosses studied has been based upon three phenotypes: rough, intermediate and smooth. The near-rough class was combined with the rough class, while the smooth-base and near-smooth classes were grouped to form a single intermediate class. This grouping suggested a 12:3:1 ratio of rough : intermediate : smooth-awned plants. The actual segregations as compared with the theoretical 12:3:1 are given in Tables II and III. The fit of the actual to the theoretical in the crosses involving Trebi and Glabron (Table II) is fairly good in some cases, but owing to the tendency to place a number of intermediate plants in the smooth-awned class, the total population shows a poor fit to the theoretical.

On the other hand, the reciprocal crosses of Trebi and Velvet, together with their total, show good fits to the theoretical (Table III). This may be explained by the fact that, as this cross was studied last, a greater familiarity with the three phenotypes had been acquired.

The F_2 data just presented suggest a two factor difference for barbing of awn. The segregation can be explained on the basis of epistasis; R is the main factor for barbing of awn; S is hypostatic to R and in the absence of R produces the intermediate condition, while the double recessive produces the smooth-awned type. On the basis of this hypothesis the F_2 plants, when tested in the F_3, should breed as follows:

TABLE II

NUMBERS OF ROUGH-, INTERMEDIATE- AND SMOOTH-AWNED PLANTS IN THE F_2 GENERATION OF RECIPROCAL CROSSES BETWEEN GLABRON AND TREBI, AS COMPARED WITH THE THEORETICAL 12:3:1 RATIO

Cross	No.	Actual			Theoretical			χ^2	P
		Rough	Intermediate	Smooth	Rough	Intermediate	Smooth		
Glabron × Trebi	6	111	21	7	104.28	26.07	8.69	1.75	0.43
	11	133	21	18	129.00	32.25	10.75	9.64	0.01
	12	170	32	16	163.50	40.88	13.63	2.60	0.28
	13	121	29	18	126.00	31.50	10.50	5.71	0.06
Trebi × Glabron	19	79	24	13	87.00	21.75	7.25	5.64	0.06
	20	158	33	14	153.75	38.44	12.81	1.00	0.61
	21	95	20	5	90.00	22.50	7.50	0.84	>0.61
	23	83	13	8	78.00	19.50	6.50	2.83	0.25
	45	136	22	10	126.00	31.50	10.50	3.68	0.16
Total		1086	215	109	1057.56	264.39	88.13	14.93	0.001

TABLE III

NUMBERS OF ROUGH-, INTERMEDIATE- AND SMOOTH-AWNED PLANTS IN F_2 GENERATION OF RECIPROCAL CROSSES BETWEEN VELVET AND TREBI, AS COMPARED WITH THE THEORETICAL 12:3:1 RATIO

Cross	No.	Actual			Theoretical			χ^2	P
		Rough	Intermediate	Smooth	Rough	Intermediate	Smooth		
Trebi × Velvet	116	59	15	8	61.50	15.38	5.13	1.72	0.44
	123	160	30	12	151.50	37.88	12.63	2.15	0.35
	124	155	37	16	156.00	39.00	13.00	less than 1	>0.61
	133	186	50	19	191.38	47.82	15.94	1.73	0.43
Velvet × Trebi	137	183	45	16	183.00	45.75	15.25	less than 1	>0.61
	146	123	22	6	113.16	28.29	9.44	3.51	0.18
	148	188	41	14	182.28	45.57	15.19	less than 1	>0.61
	151	114	23	8	108.72	27.18	9.06	1.02	0.60
Total		1168	263	99	1147.56	286.89	95.63	2.47	0.30

	F_2 genotype	F_3 behavior
rough	1 *RRSS* 2 *RRSs* 1 *RRss*	4 breeding true for rough.
	4 *RrSs*	4 segregating in the ratio 12:3:1 for rough : intermediate : smooth.
	2 *RrSS*	2 segregating 3 rough : 1 intermediate.
	2 *Rrss*	2 segregating 3 rough : 1 smooth.
intermediate	1 *rrSS*	1 breeding true for intermediate.
	2 *rrSs*	2 segregating 3 intermediate : 1 smooth.
smooth	1 *rrss*	1 breeding true for smoothness.

Three crosses involving Trebi and Glabron were given further study in the F_3. The behavior of F_3 lines from rough-awned F_2 plants is shown in Table IV.

TABLE IV

THE BEHAVIOR OF F_3 LINES FROM ROUGH-AWNED F_2 PLANTS COMPARED WITH THE THEORETICAL RATIO 4:4:2:2

Cross	No.	Non-seg.		Seg. 12:3:1		Seg. 3 rough: 1 int.-smooth		Seg. 3 rough: 1 smooth		χ^2	P
		Act.*	Theor.	Act.*	Theor.	Act.*	Theor.	Act.*	Theor.		
Glabron × Trebi	12	63	47.67	29	47.67	32	23.83	19	23.83	15.960	0.001
Trebi × Glabron	21	24	26.67	25	26.67	17	13.33	14	13.33	1.42	0.71
Trebi × Glabron	45	39	36.00	33	36.00	22	18.00	14	18.00	2.28	0.52
Total of Crosses 21 and 45		63	62.67	58	62.67	39	31.33	28	31.33	2.58	0.47

**Corrected numbers based on F_3 behavior.*

The fits are remarkably close to the theoretical in Crosses 21 and 45, but a wide deviation occurs in the case of Cross 12. It will be seen that the homozygous rough-awned lines are too numerous, whereas those segregating in the ratio of 12:3:1 are too few in number. This poor fit may be explained only as a chance variation as it would seem very unlikely that lines segregating for any degree of smoothness would be placed in the homozygous rough-awned class. The actual numbers of different segregates in F_3 lines derived from rough-awned F_2 plants show close approximations to the expected.

In Table V is shown a summary of the lines segregating for rough- : intermediate- : smooth-awned plants as compared with the theoretical 12:3:1. It is evident that good fits to the theoretical exist in all three crosses.

TABLE V

SUMMARY OF THE RATIOS OF ROUGH- : INTERMEDIATE- : SMOOTH-AWNED PLANTS IN F_3 SEGREGATING LINES, COMPARED WITH THE THEORETICAL 12:3:1

Cross	No.	—	Rough	Intermediate	Smooth	χ^2	P
Glabron × Trebi	12	Act.	879	226	85	1.76	0.47
		Theor.	892.50	223.13	74.38		
Trebi × Glabron	21	Act.	800	182	70	1.61	0.46
		Theor.	789.00	197.25	67.25		
Trebi × Glabron	45	Act.	1014	258	89	0.27	>0.61
		Theor.	1020.75	255.19	85.06		

A summary of the numbers of rough- to intermediate-awned plants in lines segregating 3:1 as compared with the theoretical numbers is given in Table VI. Satisfactory fits to the theoretical exist in the cases of Crosses 21 and 45, but only a fair approximation in the case of Cross 12. It will be noted in all three crosses that there are too many rough-awned plants at the expense of the intermediate. This discrepancy is particularly great in Cross 12.

TABLE VI

SUMMARY OF THE RATIOS OF ROUGH- : INTERMEDIATE-AWNED PLANTS IN F_3 SEGREGATING LINES, COMPARED WITH THE THEORETICAL 3:1

Cross	No.	—	Rough	Intermediate	$\frac{\text{Dev.}}{\text{P.E.}}$	Odds
Glabron × Trebi	12	Act.	969	268	4.02	142.26 : 1
		Theor.	927.75	309.25		
Trebi × Glabron	21	Act.	551	165	1.77	3.45 : 1
		Theor.	537.00	179.00		
Trebi × Glabron	45	Act.	674	200	2.14	5.38 : 1
		Theor.	655.50	218.50		

A summary of the ratios of rough- to smooth-awned plants in segregating lines as compared with a theoretical 3:1 revealed excellent fits in all three crosses (Table VII).

TABLE VII

SUMMARY OF THE RATIOS OF ROUGH- : SMOOTH-AWNED PLANTS IN F_3 SEGREGATING LINES, COMPARED WITH THE THEORETICAL 3:1

Cross	No.	—	Rough	Smooth	Dev./P.E.	Odds
Glabron × Trebi	12	Act.	536	192	1.29	1.63 : 1
		Theor.	546.00	182.00		
Trebi × Glabron	21	Act.	445	146	less than 1	< 1 : 1
		Theor.	443.25	147.75		
Trebi × Glabron	45	Act.	367	126	less than 1	< 1 : 1
		Theor.	369.75	123.25		

From the data just presented it may be concluded that the F_3 behavior of rough-awned F_2 plants supports the proposed two-factor hypothesis for the inheritance of barbed awn.

The behavior of F_3 lines from intermediate-awned F_2 plants is supplied by the data given in Table VIII. A very close fit to the expected is obtained in Cross 21. Certain deviations occur in Crosses 12 and 45 that can possibly be explained by the fact that when only small numbers of smooth-awned plants occurred in segregating lines they were overlooked, and the lines were classified as non-segregating.

TABLE VIII

THE BEHAVIOR OF F_3 LINES FROM INTERMEDIATE-AWNED PLANTS COMPARED WITH THE THEORETICAL RATIO 1:2

Cross	No.	Non-seg.		Seg. 3 intermediate : 1 smooth		χ^2	P
		Act.*	Theor.	Act.*	Theor.		
Glabron × Trebi	12	19	13.66	22	27.32	3.12	0.1–0.05
Trebi × Glabron	21	7	7.00	14	14.00	perfect fit	
Trebi × Glabron	45	13	9.00	14	18.00	2.67	0.1
Total		39	29.66	50	59.32	4.41	0.05–0.02

**Corrected numbers based on F_3 behavior.*

The total numbers of intermediate- and smooth-awned plants in segregating F_3 lines derived from intermediate-awned F_2 plants showed a satisfactory fit to the theoretical in Cross 21 but rather poor fits in Crosses 12 and 45 (Table IX). The deviations noted in the last two crosses were caused by the fact that the segregating lines contained too many intermediate- and insufficient smooth-awned plants.

TABLE IX

SUMMARY OF THE RATIOS OF INTERMEDIATE- TO SMOOTH-AWNED PLANTS IN F_3 SEGREGATING LINES COMPARED WITH THE THEORETICAL 3:1

Cross	No.	—	Inter-mediate	Smooth	$\frac{\text{Dev.}}{\text{P.E.}}$	Odds
Glabron × Trebi	12	Act. Theor.	685 642.00	171 214.00	5.04	1350.35 : 1
Trebi × Glabron	21	Act. Theor.	404 390.00	116 130.00	2.10	5.38 : 1
Trebi × Glabron	45	Act. Theor.	447 411.75	102 137.25	5.15	1350.35 : 1

It will be recalled that the F_2 material as originally classified included a near-rough class and two intermediate classes designated as smooth-base and near-smooth. As determined by F_3 behavior the plants classed as smooth-base were found to breed true for this condition, whereas those classed as near-smooth segregated into smooth-base, near-smooth and smooth types. On the basis of the two-factor hypothesis proposed for barbing of awn, the true breeding intermediate (represented by the smooth-base type) possesses the genotypic constitution *rrSS* and the segregating intermediate (represented by the near-smooth type) the genotypic constitution *rrSs*. Hence, it is apparent that the hypostatic factor *S* in the homozygous condition causes a more pronounced type of barbing than it does in the heterozygous condition.

The existence of these two intermediate types caused considerable confusion in determining the ratios within lines segregating for intermediate and smooth. Owing to environmental factors, no clear-cut line of demarcation existed between the smooth-base and near-smooth classes or between the near-smooth and smooth classes. These conditions possibly explain the deviations from the theoretical noted in Crosses 12 and 45 in regard to the numbers of intermediate and smooth-awned plants in segregating lines (Table IX).

Plants classified as near-rough in the original F_2 classification behaved either as rough or smooth-base types. Fluctuations caused by environmental factors are probably responsible for this class.

Since it has been shown that the near-rough class was based on environmental rather than on genetic differences, and since smooth-base and near-smooth classes proved to be simply the expression of different genotypes of the intermediate condition expected in the F_2 from a two-factor hypothesis based on epistasis, the grouping adopted in the F_2 appears to be correct.

The F_3 data presented support reasonably well the original hypothesis that two factors explained on the basis of epistasis are involved in barbing of awn. The results are, therefore, in agreement with those reported by Griffee (12) and Robertson, Deming and Koonce (33).

It is interesting to note that the results of this study are not in agreement with those of David (7), who previously worked with the same cross that is discussed in this paper. He reported that two-thirds of the progeny from smooth-awned plants segregated in ratios approximating 3 smooth : 1 rough. No such inverse ratio occurred in the present work. It is possible that these two investigations have been carried out with different strains of Trebi. David describes Trebi as being "six-rowed, white, high yielding, rough-awned". It is not clear in what sense he is using the term "white". If he is referring to the aleurone color, then he is definitely working with a strain of Trebi different from that employed in the present study. The aleurone color of the kernels of the Trebi parent used in this investigation was definitely bluish-gray. This description of Trebi is in accord with that given by Harlan *et al.* (15).

Earliness of Heading

Literature Review

Harlan and Martini (18) in a study of earliness in F_1 barley hybrids found that the hybrids headed quite uniformly as compared with their respective parents. In average date of emergence of awn, the hybrids tended to be intermediate between the parents. The earliness of many hybrid combinations of late varieties indicated that they contained many factors for earliness.

As a result of studying the F_3 lines of a cross between Guy Mayle × Canadian Thorpe, Neatby (30) concluded that growth period was governed by three main factors. He arrived at his conclusions by estimating the number of lines containing plants of winter habit and by comparing the standard deviations of the individual lines with the average of that of the parents. When these three factors are in a homozygous recessive condition winter habit results. Canadian Thorpe was thought to carry two factors and Guy Mayle one.

Griffee (12) found that earliness was governed by a single factor difference with early heading dominant, in a cross between Svanhals and Lion. A distribution table for date of heading of F_3 lines showed them to be of three kinds, *viz.*, early, segregating, and late. These three types appeared in an approximate 1:2:1 ratio.

David (7) studied the inheritance of earliness in the crosses Glabron × Trebi and Velvet × Trebi. The F_1 from each cross was earlier than either parent. When the F_2 plants were graphed on the basis of the number of days from planting to flowering, a ratio of nine early to seven late was obtained. A study of the F_3 lines on the basis of mean number of days from planting to flowering substantiated the view that two important complementary factors were involved in the inheritance of earliness.

Methods and Experimental Results

The F_3 lines from two F_2 populations of reciprocal crosses between Glabron and Trebi were used in a study of earliness of heading. This character was not studied in the F_2 as it was thought that a more reliable expression of the

time of heading could be obtained from studying the means of F_3 lines rather than the individual F_2 plant. A physiological character such as time of heading is so influenced by environmental factors that data pertaining to individual plants are of no great significance.

The number of plants heading out in each F_3 line was recorded in 2–3 day intervals during the heading period. The emergence of awn, combined with the splitting of the sheath of the first spike, was taken as the criterion of heading. The distribution for date of heading of the plants in each F_3 line was obtained, and from this the mean of each line for the number of days from emergence to heading was calculated.

The expression of this character was affected greatly by environmental conditions. From the behavior of the plants in parental rows, it was evident that considerable variation existed not only within individual rows but also among rows located in different areas of the field. Cool, rainy weather at heading time prolonged heading in the parental rows and in the later hybrid lines of both crosses. This condition made it impracticable to use the distribution of the parental varieties as a measure of homozygosity for the hybrid lines.

The distribution set out in two-day intervals for the mean number of days from emergence to heading, together with the coefficient of variability of parental rows and hybrid lines of Crosses 12 and 21, is given in Table X. Transgressive segregation for both earliness and lateness is clearly indicated, with both of the parental varieties occupying a central position on the distribution surface. Evidently factors for earliness were contributed by both Trebi and Glabron. The distribution of the hybrid lines shows a marked massing in the early classes which would suggest the partial dominance of early heading over late heading. The complex segregation noted in this study of earliness of heading can best be explained by a polymeric factor hypothesis.

Height of Plant

Literature Review

Vestergaard, cited by David (7), reported a segregation of 14 dwarfs to 81 normal plants in the F_2 of a cross between Binder and a dwarf-like variant. Miyake and Imai (28) crossed tall slim plants with short stout ones. The F_1 were tall and slim, whereas the F_2 segregated into a ratio of 3 tall to 1 short. Miyayawa (29) found that dwarfness tended to be dominant in a cross that he studied. A segregation of one sterile dwarf, to two sterile dwarfs, to one normal plant was obtained in the F_2. Harlan and Pope (16), on the other hand, found that a dwarf form behaved as a simple recessive to the normal barley.

Neatby (30) obtained height data on 228 F_3 lines of a cross between Guy Mayle and Canadian Thorpe. No clear indication of the number of factors involved was obtained. By comparing the standard deviations of the F_3 lines with those of the parents, Neatby concluded that at least four factors seemed to be operative.

TABLE X

DISTRIBUTION OF F_3 LINES AND PARENTAL ROWS ACCORDING TO THE MEAN NUMBER OF DAYS FROM EMERGENCE TO HEADING IN RECIPROCAL CROSSES BETWEEN TREBI AND GLABRON

Parent or cross	Classes for number of days													Mean	Coefficient of variability
	48.5	50.5	52.5	54.5	56.5	58.5	60.5	62.5	64.5	66.5	68.5	70.5	72.5		
Trebi	—	—	—	—	2	4	1	1	—	—	—	—	—	58.80	3.37 ± 0.57
Glabron	—	—	—	—	—	1	3	2	2	—	—	—	—	61.75	3.44 ± 0.58
Cross 12, Glabron × Trebi	2	2	13	19	42	27	16	18	10	6	2	3	1	58.65	7.59 ± 0.29
Trebi	—	—	—	—	3	2	—	—	—	—	—	—	—	57.30	1.91 ± 0.41
Glabron	—	—	—	—	2	3	—	—	—	—	—	—	—	57.70	1.90 ± 0.41
Cross 21, Trebi × Glabron	—	6	16	17	20	14	14	6	5	4	2	—	—	57.17	7.61 ± 0.36

David (7) studied plant height in all three generations of the crosses Glabron × Trebi and Velvet × Trebi. The F_1 plants in each cross were taller than the short parent and closely approached the height of the taller. The plants of the F_2 were much more variable than those of the F_1, and parental forms were recovered in both the F_2 and F_3 generations.

Methods and Experimental Results

The two reciprocal crosses used for the study of earliness also served as a basis for a study of plant height. The criterion of height was taken as the distance from the base of the culm to the tip of the spike, excluding awns, of the longest tiller. The plants were pulled individually and measured in inches. The variability noted in regard to earliness of heading was even more striking in the case of height of plant. This was again particularly evident in Cross 21.

The distribution of the mean heights of the progeny and parent rows of Crosses 12 and 21, together with their coefficients of variability, are given in Table XI.

TABLE XI

Distribution of hybrid lines and parent rows for mean height in inches, in reciprocal crosses between Glabron and Trebi

Parent or cross	Mean height in inches										Mean	C.V.
	27.5	29.5	31.5	33.5	35.5	37.5	39.5	41.5	43.5	45.5		
Trebi	—	—	1	5	1	1	—	—	—	—	34.00	4.88 ± 0.82
Glabron	—	—	—	—	—	—	2	1	4	1	42.50	4.71 ± 0.79
Glabron × Trebi 12	—	1	5	25	47	38	22	19	3	—	36.91	7.74 ± 0.29
Trebi	2	2	—	1	—	—	—	—	—	—	29.40	7.00 ± 1.49
Glabron	—	—	—	—	1	2	1	1	—	—	38.20	5.59 ± 1.19
Trebi × Glabron 21	3	4	27	18	30	15	6	1	—	—	34.28	8.42 ± 0.39

The parent varieties differ quite strikingly in height. Trebi averages approximately eight inches shorter than Glabron. The coefficient of variability of the F_3 lines of Cross 12 as compared with that of the parents, shows the progeny to be considerably more variable in plant height. These differences in variability are not so clear in Cross 21, since, owing to environmental factors, the parental varieties show high variability. However, in view of the fact that the parental varieties occupy the extremes of the distribution surface, while the distribution of the progeny approaches that of the normal curve, it can be concluded that a segregation of genetic factors for height has occurred. Cumulative factors appear to be involved.

Resistance to Loose Smut

Plant disease surveys have shown the loose smut disease of barley to be widespread in Canada (5, 6, 27). However, owing to low percentage infections, the estimated total loss from this disease is considered to be much less than that occurring from the covered smut disease. Loose smut is quite

common in western Canada. In 1927, the aggregate loss from loose smut occurring in Alberta has been reported as being considerable (27, 36). In Manitoba in 1930 loose smut was found in 22 fields out of 26 examined. However, the estimated damage was considered to be only one-half of one per cent (6).

It is a very significant fact that the existing smooth-awned barley varieties are susceptible to this disease. It is possible that, when smooth-awned varieties are grown to a greater extent in western Canada than they are at present, the plant disease surveys will show a correspondingly higher loss from loose smut. It is also important to note that the disease is not controlled by the simpler methods of seed treatment.

Until comparatively recently it was believed by the majority of investigators that only two distinct types of barley smut existed: covered smut (*Ustilago hordei*) causing seedling infection, and loose smut (*Ustilago nuda*) capable of causing infection only through the young embryo. Seed surface disinfectants have been advocated to control covered smut, while the hot water treatment was thought to be the only means of controlling loose smut. However, about 1923, experiments conducted with seed disinfectants showed that loose smut could be controlled in some varieties by seed treatment with formaldehyde and certain mercury compounds (45, 46). These results suggested that infection with loose smut may take place through seed-borne spores. This possibility was investigated by Tisdale and Tapke (47) who found that dehulled seed of a number of varieties, when inoculated with spores of *U. nuda* obtained from the variety Tennessee Winter, produced plants with a high percentage of loose smut.

Further studies on this type of loose smut infection by Tisdale and Griffiths (48) showed that *U. nuda* was composed of a number of variants (physiologic forms). These were found to vary not only in pathogenicity but also in the manner in which they affected the germination and development of the host plant. These investigators postulate that there are in existence strains of *U. nuda* capable only of flower infection while others appear to infect primarily through the seed. It is significant to note in this connection that Rodenhizer (34) found greater cultural differences between different forms of *U. nuda* than between *U. nuda* and *U. tritici*. More definite information in regard to possible smut strains was supplied by Tapke (43) in 1932, who found that loose smut could be caused by either of two fungi: *Ustilago nuda* (Jensen) K & S., and a new species which he has named *Ustilago nigra*. The former species was unable to cause seedling infection and could not be controlled by seed surface disinfectants. The latter species, on the other hand, caused seedling infection, and was readily controlled by Ceresan dust or liquid formaldehyde treatment of flower-inoculated seed. The spores of *U. nuda* also appeared to be considerably shorter lived than those of *U. nigra*. Tapke intimates that the divergent result obtained from seed treatment for the control of loose smut was due to a failure to recognize these two types of smut.

The existence of a smut type intermediate between *U. hordei* and *U. nuda* was discovered as early as 1894 by Biedenkopf (3). This type which he named *Ustilago medians* produced smutted spikes similar to those produced by *U. nuda*, but behaved as *U. hordei* in its manner of spore germination.

More recently Vanderwalle (49) has described a late developing smut type which he considers to be intermediate between *U. hordei* and *U. nuda*. This type was incapable of producing seedling infection.

Ruttle (35) has described five types of smut intermediate between *U. nuda* and *U. hordei*. One of these (Type 4) was found to correspond quite closely to the *U. medians* Biedenkopf and *U. nigra* Tapke. Types 2 and 3 were intermediate between Type 4 and *U. hordei*, while Types 5 and 6 were intermediate between Type 4 and *U. nuda*. Type 3 corresponded somewhat in morphological features with the late developing type of Vanderwalle. Ruttle points out that a large proportion of the smut collected in New York state was of this intermediate nature, and, while seemingly true loose smuts, were actually seedling infection and readily controlled by seed disinfectants. She states, furthermore, that spore germination is the only certain way of distinguishing between the intermediate seedling infecting and the true loose smuts.

The relation of environment to the development of the loose smut in barley appears to have received relatively little study. Seiffert, cited by Leukel (25), and Taylor and Zehner (44) found that shallow-sown seed produced less infected plants than seed sown deeply. Kirby (24) believed that soil temperatures higher than the optimum for the development of the barley seedling favored the development of loose smut. Tapke (42) studied the influence of humidity on floral infection of wheat and barley with loose smut. He concluded that relatively low humidity during the period of flowering tended to inhibit infection with loose smut in both wheat and barley. Leukel (25) found that a very wet soil (90% saturation) inhibited loose smut development somewhat, and favored its control by seed-surface dust fungicides. A very dry soil had the reverse effect. A relatively high soil temperature before emergence appeared to favor loose smut development.

In the foregoing paragraphs an attempt has been made to present a brief statement of the status, at least in America, of the loose smut disease of barley. There are in existence a number of barley smut types intermediate between *U. hordei* and *U. nuda*, both in morphological features and in their mode of infection. A number of these, while producing disease symptoms similar to those produced by *U. nuda*, are primarily seedling infecting and are amenable to control by seed disinfectants. The latter have been found to control loose smut only in certain varieties indicating that some varieties may be susceptible to the flower-infecting types of smut, while others may be susceptible primarily to the seedling-infecting types.

It is evident from the work reviewed above that the problem of loose smut in barley is a complicated one and in need of further investigation. In this connection Ruttle (35) states "further study is needed on the barley smut

types, on their field occurrence, their mode of infection, their control, their biologic forms, their host reactions, and primarily on their relationship to *U. nuda* and *U. hordei* and to one another".

There is a great need in western Canada for just such studies. Undoubtedly intermediate types of smut occur in this area, but as yet no one has separated them. It is obvious that no progress can be made in devising control measures for the loose smut disease, or in the breeding of resistant varieties, until the prevailing type and its method of infection are ascertained. Since in all likelihood the varieties to be developed in the future will be smooth-awned types, it is imperative that information regarding the loose smut disease be obtained at an early date so that the desirable combination of smooth awn and resistance to the loose smut disease can be incorporated within these varieties.

At the University of Alberta, in 1931, at attempt was made to test the reaction of the F_3 progeny of crosses between Trebi and Glabron to *U. nuda*. The method of seed inoculation as described by Tisdale and Tapke (47) was used. The inoculum used was collected from a commercial field of smooth-awned barley in the vicinity of Edmonton, Alberta. Although the inoculum showed a comparatively high percentage of germination just prior to inoculation, the resulting plants from inoculated kernels were entirely free from loose smut. It was about this time that Tapke (43) described the new species of loose smut, *U. nigra*, capable of causing seedling infection as contrasted with the true loose smut, *U. nuda*, capable only of floral infection. Examination of the inoculum used in the study reported revealed spores typical of *U. nuda*.

The results of this study serve to emphasize the need for detailed information concerning the smut fungi prevalent in western Canada, before progress can be made in breeding new varieties resistant to these diseases.

Resistance to Stripe

Literature Review

Plant disease surveys have shown that, from the standpoint of distribution and destructiveness, the stripe disease is generally less important economically than the covered smut disease (5, 6). However, periodically, owing possibly to environmental conditions, this disease becomes quite destructive. Thus in the year 1920 stripe was considered the worst disease of the barley crop in the prairie provinces (8). Sanford (36) states as a result of a disease survey of Alberta conducted in 1927 that stripe was common and at times severe. In the year 1928 barley stripe was reported as being quite prevalent in Manitoba, especially in late low-lying fields where 60% of the plants were infected (27). Twenty-five out of 108 fields examined in Alberta in 1930 showed striped plants. Four of these were classed as heavily infected with one field showing 20–30% infection (6). It is worthy of note that in the plant disease survey reports, reference has been repeatedly made to relatively high infections of stripe observed in varietal plots at experimental stations

when little was found in surrounding commercial fields. This condition may be partially due to the fact that farmers generally sow their barley at a considerably later date than is practised at experimental stations, and therefore at a time when soil temperatures are high and inhibitory to stripe development.

Methods of Infection

Until recently the stripe fungus was thought to infect plants at flowering time and to cause a systemic infection similar to that of loose smut of wheat and barley in plants from infected seed. Several studies regarding the mode of infection of this fungus have modified the general views in this regard. Ravn (32) believed that *H. gramineum*, like certain smut fungi, inhabits the growing point of the host plant and thence spreads to each young part of the plant during the formation of that part. He found that the bulk of the infection occurred at flowering time and that low percentages of infection occurred when germinated seedlings were grown in the presence of mycelium. Vogt (51) as a result of histological studies showed the mycelium inhabited the space between the glumes and the pericarp and is never found in the embryo or in the endosperm. Infection was found to take place through the coleoptile rather than the embryo. Smith (40) came to similar conclusions in that he found infection to occur while the shoot is still under the adherent glumes or during its emergence. The inoculum was found to comprise: (a) conidia lodging at the awn end of the grain, (b) mycelium penetrating from the glumes, and (c) perithecia formed inside or outside the glumes. Johnson (22) showed that it was possible to obtain artificial infection of barley simply by inoculating the germinating kernels with spores or mycelia. Dehulling of the kernel increased the infection considerably.

In a later paper, Smith (41) showed definitely how infection of barley seedlings takes place. Mycelium is considered to be the main source of inoculum and is capable of living for at least two years in the chaff and pericarp. On the germination of the kernel the hyphae infect the primary sheaths, the coleorhiza (leading to root decay) and the coleoptile (causing leaf disease). From the coleoptile each successive leaf as formed is infected—the lesions on the newly infected leaf paralleling those on the older leaves enclosing it. The uppermost infected leaf may cause external infection of the young spike. Hence it can be seen that the mode of infection is contrary to that of smut infection. That is to say, the leaves are infected first and the growing point later, whereas, in the smuts the growing point is first infected and the other parts attacked as they are formed.

Temperature Relations

Several investigators have demonstrated that low soil temperatures favor infection of barley seedlings with *H. gramineum*.

Ravn (32) found that early planting produced greater infection. Johnson (22) showed, by the use of controlled experiments, that low soil temperatures are favorable to increased infections. He found the optimum temperatures

for infection to be 10–12° C., while little occurred at soil temperatures higher than 20° C. Smith (41) points out that low soil temperatures greatly slow down germination processes, thus increasing the likelihood of infection while the shoot is still confined within the chaff. Isenbeck (21) found that soil temperatures varying from 0–8° C. with 25% soil moisture were most favorable for stripe infection. Leukel, Dickson and Johnson (26) stated that temperatures of 15° C. or less during the period of emergence favored stripe infection while the disease was inhibited by temperatures above 20° C. In a recent paper concerning the relation of temperature to the stripe diseases, Shands (37) has stated that "in analyzing the effect of temperature on the development of stripe, such reactions as the effect of temperature on the host, on the parasite and on the combination of the two in the production of disease must be studied." He points out that barley is a relatively low-temperature plant making best development at constant low soil temperatures of approximately 12–16° C. Highest stripe percentages also occurred at these constant low temperatures when the inoculated kernels were incubated at the different temperatures until they had reached approximately the same stage of development. However, when the kernels were incubated for equal periods of four days at each temperature, the greatest percentage of stripe developed at about 20° C. Change of temperature from low to high stimulated disease appearance, while the reverse change retarded disease expression.

Physiologic Specialization

Physiologic specialization in this fungus was first demonstrated by Johnson (22). A single spore isolation obtained from Edmonton, Alberta, failed to recover when transferred to room temperatures after being kept at 32°–33° C. for 10–12 days, while several other cultures tested in a similar manner recovered and grew normally. The Edmonton form also grew better at 5–6° C. than did the other cultures under test.

Christensen and Graham (4) distinguished between more than 125 races of *H. gramineum* in culture from collections obtained from 12 states of the United States and from Canada and Germany. The type of medium as well as bacterial association were found to affect races differently. Great variability in the stability of these races was noted. At least 20 races could be distinguished by their relative pathogenicity on 16 varieties of barley. Isenbeck (21) demonstrated the presence of at least three physiological forms of *H. gramineum* based on cultural characteristics and differences in pathogenicity. An American form studied proved to be less virulent than the German forms studied.

Inoculation Methods

Ravn (32) found the greater percentage of successful infections to result from floral inoculation and that low percentage of infection resulted from germinating seedlings in the presence of the mycelium.

Johnson (22) was probably the first investigator in America to demonstrate that it was possible to obtain stripe infection by seed or seedling inoculation. The period of greatest susceptibility in dehulled kernels was found to be that just subsequent to the emergence of the coleoptile.

Genau (10) found the optimum time to effect floral inoculation with conidial suspension was the day following opening, provided the temperature approximated 25° C. Young seedlings 1–2 cm. in height were found to be readily infected by means of a conidial suspension in sufficiently warm and humid atmospheres. In one experiment the plants were successfully inoculated with the ascospores of the fungus.

Fuchs (9) has devised a method of artificial inoculation consisting of the injection of a conidial suspension of the fungus into the ripe grain after soaking the latter for an hour or two in water so as to loosen the glumes from the grain and permit of inserting the needle between them. For large quantities of inoculum a modified method was devised. The barley grains are placed in a vacuum for 20 minutes in a conidial suspension in such a way that the air between the glumes and seed is removed. When the pressure is restored the conidial suspension penetrates with great impetus between the glume and the caryopsis. To stimulate abundant mycelial production, the inoculated grains are placed in an incubator at temperatures of 25–28° C. and at an atmospheric humidity of 90–100%. It is claimed that this method gave results in complete agreement with the floral method used by Genau (10).

Isenbeck (21) made a thorough study of inoculation technique in regard to the stripe disease. He investigated five infection methods as follows:

1. Floral inoculation using a wet conidial suspension.
2. Floral infection using dry conidia.
3. Seedling inoculation.
4. Grain inoculation using spores.
5. Grain inoculation using mycelia.

In both types of floral inoculations the inoculum was introduced between the flowering glumes and palea of each individual flower. The dry method gave consistently higher stripe infection than the wet method. Generally, floral inoculations gave uncertain results in that they were readily influenced by environmental factors.

Seedling inoculation, after being fully investigated, was discontinued owing to the fact that the resulting disease symptoms were distinctly abnormal.

Dehulled kernels were used in both methods of grain inoculations. The spore method consisted of rolling the kernels while damp in spore dust, while in the mycelium method the kernels were mixed with small pieces of agar containing mycelium and later laid out in Petri dishes to germinate. The grain inoculations resulted in high infections and generally gave much more certain results than floral inoculations. Only 4–5 weeks were necessary for testing when seed inoculation was practised, while over a year was necessary in the case of floral inoculations.

Shands (37) obtained relatively high infections of stripe when plants were enclosed in glassine bags just prior to heading and sprayed with a conidial suspension of the fungus. Seed inoculations of dehulled kernels, effected by placing them between layers of potato dextrose agar on which the fungus was growing and incubating overnight before planting, also gave good infections.

Genetic Studies

Isenbeck (21) studied the reaction to *H. gramineum* of the progeny of eight crosses involving different combinations of immunity, resistance and susceptibility in the parents. Flowers of F_2 plants were inoculated thus giving ratios for the F_3. The F_4 was also studied in some cases. All the crosses were tested by the method of wet infection, two by dry infection and one F_3 was also tested in the greenhouse by the infection of the grain. Generally immunity was found to be dominant; crosses between two immune varieties gave no susceptibles, whereas crosses between two resistant types resulted in some susceptible types. Transgression also occurred when two susceptibles were crossed. The results can only be explained by assuming a large number of factors.

Shands *et al.* (38) studied stripe reaction in the progeny of a number of crosses between Oderbrucker, a white rough-awned variety and Leiorrhynchum, a small black smooth-awned variety. A wide range of variation from susceptible to highly resistant selections was found. Back crossing to the Oderbrucker parent resulted in increased susceptibility to the disease. The resistance of smooth-awned selections under field conditions was confirmed by results obtained from artificial inoculations. Stripe development was found to be readily influenced by environmental factors. Their results, while showing the heritable nature of stripe reaction, fail to give any indication of the number of factors involved.

Methods and Experimental Results

A method of floral inoculation, using a wet conidial suspension, was utilized in the present inheritance studies. The inoculum consisted, in the main, of diseased leaves of badly infected plants from a plot of Canadian Thorpe; this was supplemented from time to time by a composite sample of inoculum from the varietal plots. The infected leaves were placed in an incubating chamber overnight to stimulate spore production. The following day the leaves were rubbed together under water until they were disintegrated. The spore suspension was then strained through three layers of cheesecloth to remove all suspended vegetative matter. Two spikes from approximately 500 F_2 plants of crosses involving Trebi and Glabron, as well as two spikes of 100 parental plants, were selected at random for inoculation. This consisted of dipping the spikes into the solution and immediately enclosing them in glassine bags. The spikes were given additional sprays at four to five day intervals by means of an atomizer, the nozzle of the latter being inserted through slits cut in the glassine bags. The weak straw of the barley plant

caused considerable difficulty in that it was necessary to support the majority of the bagged spikes. Even with this precaution a number of spikes were broken over by the wind and rain.

The kernels from the inoculated spikes were sown as early in the spring of 1932 as was deemed practicable with soil temperatures ranging from 10–13° C. The infected plants of both hybrid and parental lines showed the

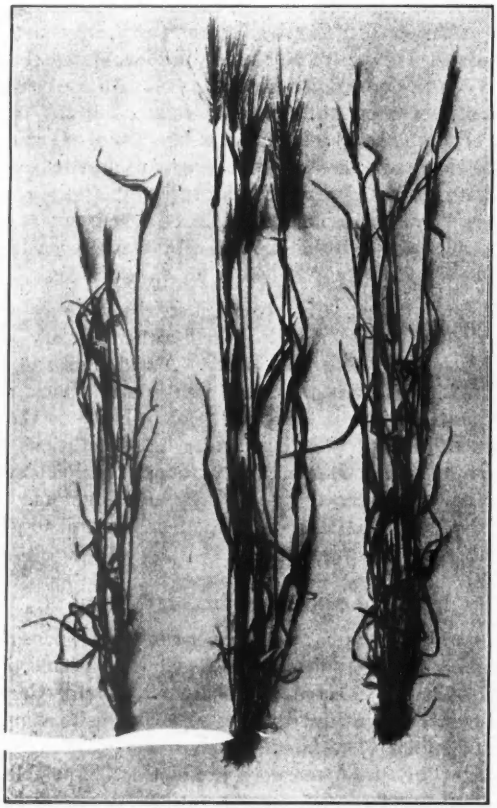

FIG. 2 *Barley plants infected with stripe, Helminthosporium gramineum. Normal plant in the centre. (Photograph taken when normal plants were approaching maturity).*

characteristic striping of the disease just prior to heading. Little or no partial infection was evident, all infected plants being ruined economically (See Fig. 2). The infection classes in percentage for the parental rows and F_3 hybrid lines are given in Table XII.

TABLE XII

REACTION OF F_3 LINES AND PARENTAL VARIETIES TO *H. gramineum* IN RECIPROCAL CROSSES OF GLABRON AND TREBI

Cross or parent	Infection classes in percentage								
	0	3	8	13	18	23	28	33	58
Trebi	39	8							
Glabron		8	14	12	7	5	1		
126 lines, Glabron × Trebi	36	40	31	13	3	—	1	1	1
336 lines, Trebi × Glabron	137	93	48	20	11	5	1	—	—
Total, 462 lines	173	133	79	33	14	5	2	1	1

The Trebi parent appears to be highly resistant to the form of the pathogen (*H. gramineum*) used, while Glabron shows infection varying from 3% to 28%, with the greatest number of lines showing from 8% to 13% infection. The wide range of infection exhibited by Glabron would indicate certain limitations in the inoculation methods employed. There is no evidence of any transgression toward greater susceptibility in the hybrids except in one line, which gave 58% infection and to which no particular significance can be attached. Isenbeck (21) found that the number of resistant individuals in the progeny increased with the degree of resistance of the two parents. The present results bear out this conclusion. Keeping the parental types in view, it is extremely difficult to postulate any definite mode of inheritance. It is important to point out, at this time, that the plants selected in the F_2 for inoculation were really a random distribution of the early plants, since there had been a tendency to select such plants for inoculation purposes. This may have possibly influenced the results obtained.

The results of this study serve to emphasize the need, when studying the reaction of a hybrid population to infection with *H. gramineum*, of inoculation methods that will give relatively certain results. The wide range of infection percentages exhibited by the Glabron parent indicates that, in the method of inoculation employed, the percentages of kernels successfully infected per inoculated spike varied considerably. Isenbeck (21), after having made a careful study of a number of inoculation methods stated . . . . "It is evident that the method of flower infection, even if thoroughly worked out, will never be as reliable as a method of grain infection, the whole of which can be carried out in the greenhouse under strictly controlled uniform conditions. Flower infection is dependent upon so many factors which cannot be controlled or even known, that a high degree of certainty of infection is hardly possible, especially in the very delicate work with *Helminthosporium*."

The same worker concludes that for varietal tests where only a comparative stripe reaction is desired, inoculation by the dry spore dust (field infection) should be practised, as this method most nearly approaches that occurring

under natural conditions. On the other hand, when absolute infection percentages are desired as in genetic studies, greenhouse infections, involving kernel inoculation by either spore dust or mycelia, should be used.

Correlation Studies

In an endeavor to obtain further information regarding the nature of the inheritance of stripe reactions, simple correlations were calculated between stripe infection percentages and both mean height of plant and mean number of days from emergence to heading; also a test of independence or association (11) was made between barbing of awn and stripe reaction. Mean height of plant was correlated with mean number of days from emergence to heading. The correlation coefficient values obtained in this study are summarized in Table XIII.

TABLE XIII

CORRELATION VALUES OBTAINED BETWEEN CHARACTERS OF F_3 HYBRID LINES OF RECIPROCAL CROSSES BETWEEN GLABRON AND TREBI

Characters correlated	Cross number	Correlation coefficient	P
Mean height and per cent stripe infection	12 and 21	0.388	0.05
Mean number of days to heading and per cent stripe infection	12 and 21	0.124	greater than 0.50
Mean height of plant and	12	0.728	less than 0.01
mean number of days to heading	21	0.737	less than 0.01

High significant correlations were demonstrated between mean height of plant and mean number of days from emergence to heading. Although this association may be partially explained on a purely physiological basis, its high value indicates that certain of the factors responsible for earliness of heading also determine plant height. No significant correlation was shown between mean number of days to heading and percentage stripe infection.

The value $r=0.388$ obtained between mean height of plant and per cent stripe infection is statistically significant in the light of its P value, .05. As tallness of plant and susceptibility to the stripe disease entered the cross together, this value may indicate a slight genetic linkage.

A test of independence or association calculated between barbing of awns and per cent stripe infection gave a P value of 0.27 which is indicative of a lack of association between these characters.

A number of individual plant selections were made, based on stripe resistance, smoothness of awns, earliness of maturity and strength of straw. These are now being compared with the standard varieties in the regular varietal test plots.

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STUDIES OF TOTAL ERYTHROCYTE AND LEUCOCYTE COUNTS OF FOWLS

IV. ERYTHROCYTE AND LEUCOCYTE COUNTS OF BIRDS RAISED IN CONFINEMENT[1]

BY E. I. PALMER[2] AND JACOB BIELY[3]

Abstract

Erythrocyte and leucocyte counts were made of 65 S.C. White Leghorn females and 50 S.C. White Leghorn males which were raised in confinement from the age of one day until sexual maturity.

The mean erythrocyte count of the males was significantly higher than the mean of the females. There was no significant difference between the mean leucocyte counts of the males and the females. The erythrocyte counts of the confined birds were significantly lower than those of normal birds kept under natural conditions and those of one- to two-day-old chicks, while the leucocyte counts were significantly higher.

In a previous paper (1) the authors have reported on the total number of erythrocytes and leucocytes of 100 single-comb White Leghorn adult females and 47 one- to two-day-old single-comb White Leghorn chicks. The mean erythrocyte and leucocyte counts of the adult birds were 2,782,000 $\pm$ 22,580 and 32,150 $\pm$ 450 respectively; while the mean erythrocyte and leucocyte counts of the chicks were 2,480,000 $\pm$ 3176, and 20,600 $\pm$ 286 respectively. The difference in the erythrocyte and leucocyte counts of the two groups of birds was in each case statistically significant.

The prevailing tendency to raise young and growing chicks in confinement suggested an investigation of the effects of confinement on the total erythrocyte and leucocyte counts of fowls. While it was originally intended to make bi-weekly counts of chicks from one to two days of age to maturity, the data reported herein are based only on one series of counts.

Material and Methods

The birds used in this investigation consisted of 115 single-comb White Leghorn birds of which 65 were females and 50 males. From one day old to about ten weeks the chicks were raised in battery brooders. They were then transferred to large wire cages where they were kept until they began to lay. The battery brooders and the cages were kept in a large, well ventilated room. No direct sunlight reached the chicks throughout the period of confinement.

[1] *Manuscript received July 13, 1935.*
Contribution from the Department of Poultry Husbandry, University of British Columbia, Vancouver, Canada.

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An all-mash ration and water were continually available to the birds. No other feed was given. During and up to the time when the blood counts were made ($3\frac{1}{2}$ to 5 months) the birds appeared to be healthy and in excellent condition.

The counts were performed according to the technique reported in a previous paper (4). In the case of several birds, duplicate and triplicate counts were made.

Experimental

Data regarding the total erythrocyte and leucocyte counts of 65 females and 50 males are shown in Tables I and II. It will be seen that the mean erythrocyte count of the males was significantly higher than that of the

TABLE I

STATISTICAL ANALYSIS OF THE ERYTHROCYTE COUNTS OF BIRDS RAISED IN CONFINEMENT, NORMAL ADULT FEMALES AND ONE- TO TWO-DAY-OLD CHICKS

	(1) 65 females kept in confinement	(2) 50 males kept in confinement	(3) 100 normal females	(4) 47 1–2-day-old chicks
Mean erythrocyte count	2164600 ± 24406	254800 ± 25186	2782000 ± 22580	2480000 ± 31576
Standard deviation	291646 ± 17255	264000 ± 17806	334777 ± 15969	318338 ± 22366
Coefficient of variability	13.47 ± 1.79	10.36 ± 0.69	12.03 ± 0.58	12.83 ± 0.91

Difference of the means; *Columns 1 and 2: 383400 ± 35071 or 10.93 × P.E.*
Columns 1 and 3: 716400 ± 33249 or 18.56 × P.E.

TABLE II

STATISTICAL COMPARISON OF THE LEUCOCYTE COUNTS OF BIRDS RAISED IN CONFINEMENT, NORMAL ADULT FEMALES AND ONE- TO TWO-DAY-OLD CHICKS

—	(1) 65 females kept in confinement	(2) 50 males kept in confinement	(3) 100 normal females	(4) 47 1–2-day-old chicks
Mean leucocyte count	41700 ± 1021	37400 ± 1007	32150 ± 450	20600 ± 286
Standard deviation	12205 ± 722	10558 ± 712	6680 ± 318	2889 ± 202
Coefficient of variability	29.26 ± 1.86	28.23 ± 2.03	20.77 ± 1.029	14.02 ± 0.99

Difference of the means; *Columns 1 and 2: 4300 ± 1434 or 2.99 × P.E.*
Columns 1 and 3: 9550 ± 1116 or 8.55 × P.E.
Columns 2 and 3: 5250 ± 1103 or 4.76 × P.E.

females. There was no significant difference, however, in the leucocyte counts of the female and male birds. This is in agreement with the results obtained by other investigators. The frequency distribution of the erythrocyte and leucocyte counts is shown in Tables II and III.

A statistical comparison of the present data with those obtained in a study of 100 normal adult females kept under natural conditions and 47 one- to two-day-old chicks, reported in a previous investigation (1) is shown in Tables

TABLE III

FREQUENCY DISTRIBUTION OF THE ERYTHROCYTE COUNTS OF BIRDS RAISED IN CONFINEMENT

No. of erythrocytes (000)	Under 1699	1700–1899	1900–2099	2100–2299	2300–2499	2500–2699	2700–2899	2900–3099	3100–3299
% of total birds (65 females)	3.06	4.59	19.89	27.54	29.07	9.18	3.06	1.53	1.53
% of total birds (50 males)				10	24	20	26	18	2

TABLE IV

FREQUENCY DISTRIBUTION OF THE LEUCOCYTE COUNTS OF BIRDS RAISED IN CONFINEMENT

Number of leucocytes	Under 19999	20000–24999	25000–29999	30000–34999	35000–39999	40000–44999	45000–49999	50000–54999	55000–59999	60000–64999	65000–69999	Over 70000
% of total birds (65 females)	1.53	3.06	6.12	18.36	19.89	24.48	6.12	9.18	1.53	4.59	3.06	1.53
% of total birds (50 males)		10	16	24	16	10	10	6	4	4	—	—

I and II. It will be seen that the erythrocyte counts of the 65 females that were raised in confinement were lower than those of the normal adult females or the one- to two-day-old chicks. The difference in the respective means is statistically significant, being over 3.5 times the probable error of the difference. On the other hand, the leucocyte counts of the females and males raised in confinement were significantly higher than those of the normal adult females and the one- to two-day-old chicks. The difference in the respective means is statistically significant.

Discussion

Since the discovery of Vitamin D and the role which it plays in the mineral metabolism of the fowl it is no longer difficult to raise and keep birds in confinement. In fact, with the development of the so-called complete rations and of the laying batteries the whole life span of a chicken can be successfully passed in confinement.

The physiological aspects of the effects of confinement on birds do not appear to have been given due consideration as yet. Recently Buckner *et al.* (2) made some very interesting observations on the subject. They report that White Leghorn cockerels raised in confinement in battery brooders, from which direct sunlight was excluded, developed abnormally large combs and smaller testes than those raised in colony brooders with access to a grass range and receiving direct sunlight. Moreover, recently Jungherr (3) has reported what he considers an important point, that "the absolute and relative lymphatic character of the blood of birds kept under laboratory conditions becomes markedly accentuated." Thus he reports that the average leucocyte count of a group of birds kept under laboratory conditions

was 28,816 per cu. mm. when the birds were one month old, 34,962 at two months, and 38,591 at three to four months. Jungherr's data would appear to be in general agreement with the data reported in this investigation.

Much more extensive data than are presented here, however, would have to be gathered before the effects of confinement on total erythrocyte and leucocyte counts could be definitely ascertained. Considering the conditions under which the present series of birds was raised (strict confinement, lack of sunlight, lack of sufficient exercise, etc.), it is not surprising that the erythrocyte and leucocyte counts were affected. The increased number of the leucocytes would tend to indicate that certain factors mitigated against the normal physiological functions of the birds. The increase in leucocytes might be due to (i) mechanical friction resulting from confinement to wire cages; (ii) continual feeding; (iii) incomplete digestion of food; and (iv) bruises. In connection with the latter it may be noted that some of the birds, particularly the males, developed breast blisters. On the whole the leucocyte counts of these birds were not higher, however, than those of birds which did not have breast blisters.

The effect of continuous confinement on the total erythrocyte and leucocyte counts would seem to be of more than passing interest. It is hoped that this preliminary study will stimulate a more comprehensive study of the physiology of confined birds.

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STUDIES ON THE ENDOPARASITIC FAUNA OF TRINIDAD MAMMALS

I. SOME PARASITES OF TRINIDAD DEER[1]

By Thomas W. M. Cameron[2]

Abstract

Four new species of nematodes, referred to three new and one known genera, are described from the deer, *Mazama simplicicornis*, from Trinidad, B.W.I.

While in Trinidad in the early part of 1935, the writer had the opportunity of examining a number of mammals for internal parasites. Owing to lack of time, it was possible to collect only a few species personally, but thanks to the generous co-operation of Professor F. W. Urich of the Imperial College of Tropical Agriculture, a number of animals was secured subsequently and the entrails, after being fixed in formalin, were forwarded to the Institute for dissection and examination. In this way a considerable amount of parasitic material was obtained. The unwanted parts of the carcasses, foetuses, etc., were distributed to other laboratories and museums.

Faunistically, Trinidad is part of the South American Continent, and although local races of animals are recognized in some cases by some mammalologists, there are few, if any, species there which do not also occur in Venezuela. The island, however, is sufficiently far distant from the mainland to prevent the ready exchange of mammals, with the exception of bats, and so the opportunity has been taken to study the parasites of an island community.

Parasites of Deer

A single species of deer—*Mazama simplicicornis*—occurs in Trinidad. This deer was present when the island was discovered by Columbus and, although other species have been introduced to other West Indian islands (such as Tobago and Barbuda) from South America and Europe, there is no history of such an introduction into Trinidad. One is probably justified in assuming, accordingly, that the parasites of these animals have been present since the deer became inhabitants of the island. There are no other ruminants on the island except domesticated sheep, goats, cattle (including zebu) and buffalo of Indian origin. Unfortunately in the short time at my disposal, I was only able to secure a single animal. However, it proved to contain four species of nematode worms of the family Trichostrongylidae, all of them in the small intestine. All appear to be new to science. Fragments of a cestode, closely resembling, if not identical with *Moniezia benedeni* were also seen. In the absence of complete worms however, a more definite identification is impossible.

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Contribution from the Institute of Parasitology, McGill University, Macdonald College, Que., with financial assistance from the National Research Council of Canada.
[2] *Research Professor of Parasitology, McGill University; and Director, Institute of Parasitology, Macdonald College, P.Q., Canada.*

1. *Nematodirus urichi* SP. NOV.

Three females and a single male of this species were recovered. The average length of the females is 18.5 mm., and the length of the male is 10.5 mm.

The cuticle is finely striated both transversely and longitudinally, the transverse striations being about 0.005 mm. apart, while the longitudinal striations are even finer. There are about 20 secondary, more prominent, longitudinal ridges, 0.02 mm. apart: a strip, 0.12 mm. wide on either side of the lateral lines, is free from these ridges. The cuticle on the head of the single male is inflated to form a sphere 0.05 mm. in diameter; the head cuticle of the females, however, is not inflated and is thinner than on the rest of the body. The mouth opening in both sexes is surrounded by a leaf-crown of very minute triangular elements, outside of which lie six circum-oral papillae. Two very small cervical papillae are present about 0.05 mm. from the anterior end.

The oesophagus is of the simple club-shaped type, about 0.6 mm. long in the female. It is 0.075 mm. wide at its maximum diameter but it is slightly swollen at its anterior end. A single large anterior oesophageal tooth is present. (Fig. 1). The nerve ring is situated about the junction of the anterior and middle third and the excretory pore lies at the level of the junction of the oesophagus and intestine.

Unlike the species of *Nematodirus* found in domestic ruminants in temperate climates, the tail of the *female* is *not* truncated but is prolonged into a conical tip. Anterior to the anus, which is 0.125 to 0.15 mm. from the tip of the tail, (Fig. 2) the width of the body quickly increases to a diameter of 0.2 mm.

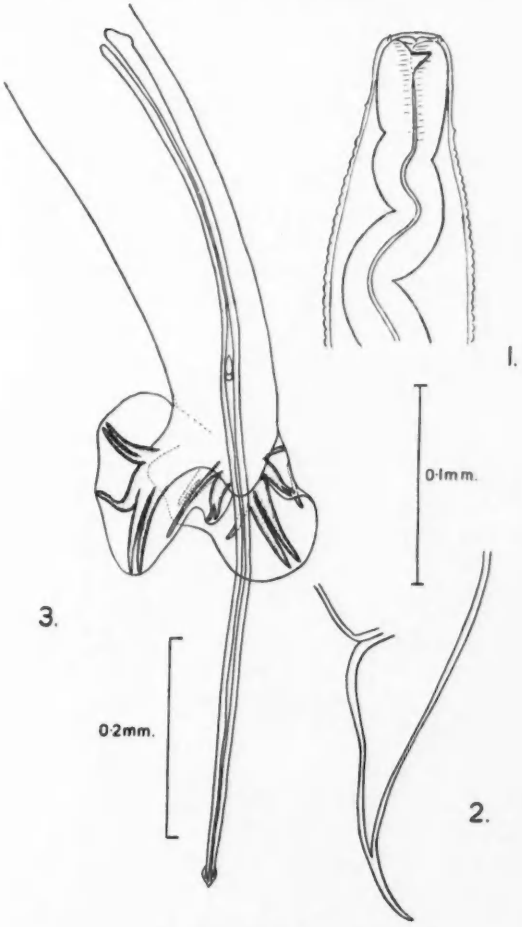
Both genital tubes originate in the anterior part of the body but the posterior ovary passes backwards to just in front of the anus. There it turns on itself and almost immediately becomes transformed into uterus. The two uteri are consequently opposed to each other. The genital tubes make several coils round the intestine. The ovejector is of the usual type with each arm 0.5 mm. long with a central "bulb" and a short common vagina which opens about 8 mm. from the tail—just posterior to the middle of the body. The eggs are of the large *Nematodirus* type, with a relatively thick shell, and measure 0.15 mm. by 0.08 mm. They have reached the eight-cell in the ovejectors.

The male has a typical *Nematodirus* type of bursa (Fig. 3) with two large lateral lobes. The dorsal lobe is split into two parts, each being fused with the corresponding lateral lobe.

The dorsal ray is also completely split, each element ending in two digitations. The externo-dorsal ray is long and slender, running parallel with, but quite separate from the postero- and medio-lateral rays and terminating about midway between their tips and the junction of the dorsal and lateral lobes. The postero- and medio-lateral rays are parallel to each other. The ventral rays are also parallel with each other and are situated at right angles

to these two lateral rays. The externo-lateral lies almost mid-way between these two pairs but, unlike them it does not quite reach the edge of the bursal membrane. The central part of the medial surface of each lateral lobe of the bursa is covered with numerous oval swellings.

The spicules are long and filiform, measuring 0.9 mm. in length. They are fused at their tip to form a heart-shaped termination, which is constricted at the base and in which each spicule bifurcates. A very small, transparent gubernaculum is present. Caudal papillae are absent.



Nematodirus urichi SP. NOV.

FIG. 1. *Head of female.* FIG. 2. *Tail of female.* FIG. 3. *Tail of male.*

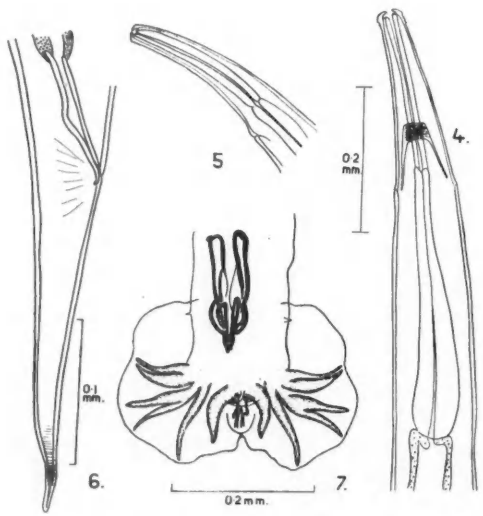
This species differs in several important respects from other members of the genus, notably in the head region and the tail of the female. The name *Nematodirus urichi* sp. nov. is accordingly proposed for it. It may be distinguished from other known species by the leaf-crown of minute elements and by the pointed tail of the female.

II. *Mazamastrongylus trinitatis* GEN. ET SP. NOV.

Several females and two males of this species were obtained. The average length of the females was 7.7 mm. and of the males, 6 mm.

No transverse striations are visible on the cuticle except on the head of both sexes and on the tail of the female. There are, however, about 20 prominent longitudinal striations which are not quite continuous and somewhat variable in number. Between these are numerous very fine longitudinal striations.

The cuticle of the head region is not inflated but the actual anterior end is distinctly dome-like with a central mouth opening surrounded by six small circum-oral papillae. Immediately behind this are some half-dozen rather coarse transverse striations, which are more prominent in the female than in the male. (Figs. 4 and 5). The oesophagus is divided into two distinct parts. The anterior part is 0.2 mm. in length and 0.025 mm. in width while the posterior part is 0.4 mm. long with a maximum width of 0.075 mm. The dimensions are similar for both sexes.



Mazamastrongylus trinitatis GEN. ET SP. NOV.

FIG. 4. *Head of female (dorsal view).* FIG. 5. *Head of male (lateral view).*
FIG. 6. *Tail of female.* FIG. 7. *Tail of male.*

Two small cervical papillae are present at the level of the junction between the two parts of the oesophagus. The nerve ring surrounds the posterior portion of the tubular anterior part. The excretory pore is prominent and is situated just posterior to the cervical papillae.

The female has a long, somewhat bluntly pointed tail, 0.24 mm. in length. (Fig. 6.) Just anterior to the tip, which is covered with minute spines, there is a number of somewhat coarse transverse striations; these become gradually finer, to disappear completely about a quarter of the distance from the anus. Anterior to the anus, the body rapidly increases to its maximum width of 0.16 mm.

Both genital tubes originate in the middle region of the body. The anterior tube passes forward, turns about 2 mm. from the head, and passes directly backwards. The posterior tube passes backwards almost to the anus when it turns forward again. Both tubes are slightly undulating but neither actually encircles the intestine. The two uteri are opposed and relatively long. As a rule a single row of eggs lies in them with their longitudinal axes across the body, but occasionally two eggs lie abreast in the other direction. The ovejectors are short but massive and are divided into two *pars muscularis*, each 0.15 to 0.2 mm. long and a common, very thick *pars ejectrix* 0.2 mm. long. The vagina is very short and the vulva is an unprotected slit about 1.5 mm. from the tail, dividing the body in the ratio of 4 : 1.

The eggs are thin-shelled and oval and measure 0.07 mm. by 0.05 mm. They had reached the morula stage at the end of the uteri.

The male bursa has two large lateral lobes and a small dorsal lobe (Fig. 7). The dorsal ray is short and stout and bifurcated for about half its length. Each bifurcation carries three digitations, the two medial being long and pointed while the other is shorter. The externo-dorsal ray is stout and curved. It is considerably shorter than the postero-lateral ray, which is separated from the other two lateral rays. The medio- and externo-lateral rays lie close together but their tips diverge, the externo-lateral being the stouter. The ventral rays are relatively large and are separated from the lateral group. They are directed away from them and are parallel to each other throughout their entire length.

An incipient dorsal lobe, supported by two weak curved rays, is present within the main bursa, anterior to and ventral of the main dorsal lobe.

Small pre-bursal papillae are present at the junction of bursa and body.

The spicules are similar, short and irregular in outline. They measure about 0.16 mm. long. They have four points. Ventrally there is a blunt club-shaped termination directed somewhat laterally. In the medial plane there are two sharp points, one straight and one undulating. In both specimens, the undulating points of both spicules were in close apposition to give a spear-shaped tip. Dorsally there is a shortish, blunt point ending in a definite, but irregular knob. No accessory pieces could be seen.

The seminal vesicle extends for about half the body length. The testicular tube is almost straight and originates about 0.75 mm. posterior to the mouth opening.

This parasite shows affinities to both *Ostertagia* and to *Cooperia* but differs from both in several important respects. Notably, it differs in the division of the oesophagus into two parts. The writer has been unable to assign it to any known genus and accordingly the new name *Mazamastrongylus trinitatis* gen. et sp. nov. is proposed for it. It may be distinguished by the division of the oesophagus into two parts, presence of cervical and pre-bursal papillae and of an accessory bursal membrane, and its *Cooperia*-like head.

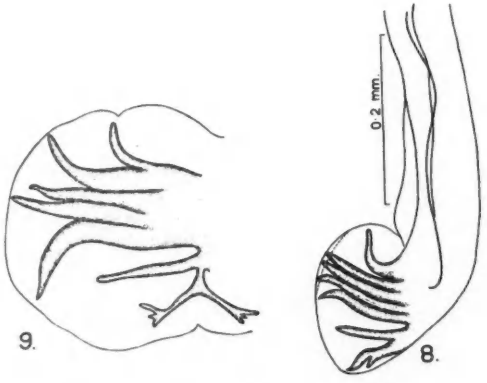
III. *Ierestrongylus filiformis* GEN. ET SP. NOV.

Three males but no females of this species were found, in a poor state of preservation. They have an average length of 6.5 mm.

The cuticle is finely striated transversely with about 20 longitudinal striations as well. There is a conspicuous ventral crest present which runs from just behind the head to the origin of the bursa.

In two of the specimens the cephalic cuticle is distinctly inflated; in the third it has a terminal cap closely resembling that of *Cooperia*. The mouth opening is simple and surrounded by six small papillae. The oesophagus is of the simple claviform type, 0.35 mm. long. Cervical papillae are small and situated about half way down its length. The excretory pore is situated just anterior to the oesophageal-intestinal junction.

The bursa (Figs. 8 and 9) is relatively voluminous with two large lateral lobes separated from each other by a small dorsal cleft; there is no dorsal lobe. The edge of the bursa is "scalloped" and there are numerous small projections on its inner surface.



Ierestrongylus filiformis GEN. ET SP. NOV.

FIG. 8. *Tail of male (lateral aspect).* FIG. 9. *Diagram of bursa (ventral view).*

The dorsal ray is divided throughout most of its length, one part lying in each lateral lobe. Each bifurcation terminates in three digitations, the medial of which is very small and the ventral recurved.

The externo-dorsal ray is relatively stout and lies midway between the lateral group and the dorsal ray. It does not reach the edge of the bursa. The three lateral rays and the latero-ventral are about the same size and lie parallel with each other for most of their length. The outer members of the group however, curve outwards at their tips whereas the two central ones are more or less straight. The ventro-ventral ray is smaller than the others and curved anteriorly.

No pre-bursal papillae were seen. The spicules are equal, long and filiform, measuring 0.65 mm. in length. The tip of each is bifid. No accessory pieces were observed.

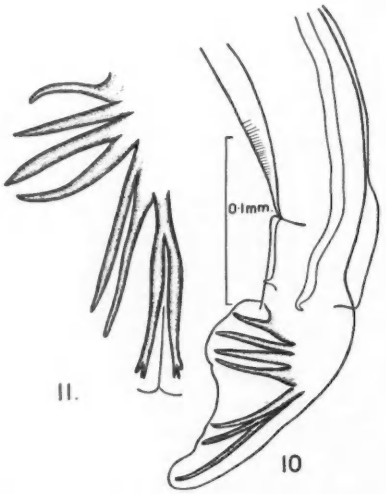
It is realized that it is unsatisfactory to describe a new species on the basis of males alone, but these males differ so distinctly from any previously recorded from ruminants, that it seems desirable to put their description on record. Unfortunately, without females, it is impossible to refer them to their proper sub-family although their appearance suggests that they belong to the Heligmosominae rather than to the Trichostrongylinae. The bursal formula somewhat recalls that of the genus *Trichostrongylus*, but it differs in the long filiform spicules, the absence of a gubernaculum, and the presence of a ventral crest. These points, even in the absence of the female, seem to justify the creation of a new genus and the name *Ierestrongylus filiformis* gen. et sp. nov. is proposed for it.

IV. *Mazamanema longibursatum* GEN. ET SP. NOV.

A single male, in a poor state of preservation, was obtained. It measures 2.3 mm. in length with a maximum breadth of 0.075 mm. It was lying in a flat spiral when collected but was easily extended. The cuticle is finely striated transversely and has about 14 faint longitudinal ridges and a small ventral crest. The head is simple and rounded with six inconspicuous circumoral papillae. There are two obvious cervical papillae, 0.05 mm. from the anterior end. The oesophagus is simple and club-shaped, nearly 0.3 mm. in length.

The bursa is long and without a dorsal lobe. (Fig. 10). There is, however, a deep dorsal cleft between the two lateral lobes. The dorsal ray is very long (Fig. 11) and split for nearly its entire length, each bifurcation terminating in three small digitations. The main arms of this ray are almost parallel with each other throughout their entire length. The externo-dorsal ray is long and thin and diverges only slightly from the dorsal ray. The postero-lateral ray is similar and is situated in close proximity to the externo-dorsal and widely separated from the other lateral rays. These, together with the two ventral rays, are situated in a group at right angles to the main axis of the body. The two laterals and the latero-ventral are thin and somewhat

elongated, whereas the ventro-ventral is considerably shorter. Small pre-bursal papillae are present just in front of the origin of the bursa and just in front of them is a distinct ventral groove at the termination of the ventral crest. The spicules are equal, long and filiform, each measuring 0.22 mm. Each ends in a single point, which is somewhat hook-shaped. No accessory pieces were seen.



Mazamanema longibursatum GEN. ET SP. NOV.

FIG. 10. *Tail of male (lateral aspect).* FIG. 11. *Diagram of bursa (ventral view).*

The remarks on the preceding species apply even more strongly to this form. The male differs, however, from all known members of the family by the possession of a ventral crest, a pre-bursal groove, long slender papillae and by the bursal formula which is characterized by the elongated split dorsal ray, with the externo-dorsal and postero-lateral close to it, and by the grouping of the other four rays distant from them.

The name *Mazamanema longibursatum* gen. et sp. nov. is accordingly proposed for it.

Acknowledgment

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Reference

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